

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
29 August 2002 (29.08.2002)

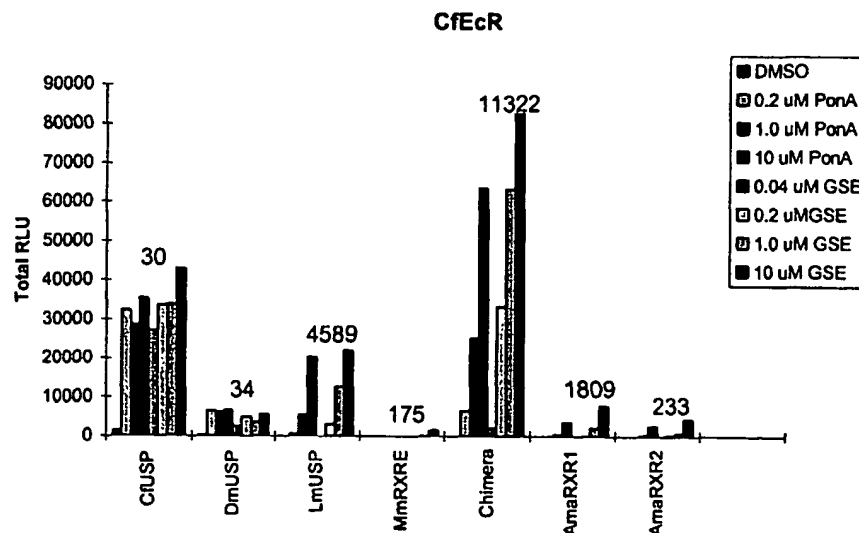
PCT

(10) International Publication Number  
**WO 02/066614 A2**

- (51) International Patent Classification<sup>7</sup>: **C12N**
- (21) International Application Number: **PCT/US02/05706**
- (22) International Filing Date: 20 February 2002 (20.02.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/269,799 20 February 2001 (20.02.2001) US  
60/294,814 31 May 2001 (31.05.2001) US  
60/294,819 31 May 2001 (31.05.2001) US
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- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent

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(54) Title: CHIMERIC RETINOID X RECEPTORS AND THEIR USE IN A NOVEL ECDYSONE RECEPTOR-BASED INDUCIBLE GENE EXPRESSION SYSTEM



(57) Abstract: This invention relates to the field of biotechnology or genetic engineering. Specifically, this invention relates to the field of gene expression. More specifically, this invention relates to a novel ecdysone receptor/chimeric retinoid X receptor-based inducible gene expression system and methods of modulating gene expression in a host cell for applications such as gene therapy, large-scale production of proteins and antibodies, cell-based high throughput screening assays, functional genomics and regulation of traits in transgenic organisms.

WO 02/066614 A2



(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
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BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

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- of inventorship (Rule 4.17(iv)) for US only

**Published:**

- without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

**CHIMERIC RETINOID X RECEPTORS AND THEIR USE IN A NOVEL ECDYSONE  
RECEPTOR -BASED INDUCIBLE GENE EXPRESSION SYSTEM**

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**FIELD OF THE INVENTION**

This invention relates to the field of biotechnology or genetic engineering. Specifically, this invention relates to the field of gene expression. More specifically, this invention relates to a novel ecdysone receptor/chimeric retinoid X receptor-based inducible gene expression system and methods of  
10 modulating the expression of a gene within a host cell using this inducible gene expression system.

**BACKGROUND OF THE INVENTION**

Various publications are cited herein, the disclosures of which are incorporated by reference in  
15 their entireties. However, the citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

In the field of genetic engineering, precise control of gene expression is a valuable tool for studying, manipulating, and controlling development and other physiological processes. Gene expression is a complex biological process involving a number of specific protein-protein interactions.  
20 In order for gene expression to be triggered, such that it produces the RNA necessary as the first step in protein synthesis, a transcriptional activator must be brought into proximity of a promoter that controls gene transcription. Typically, the transcriptional activator itself is associated with a protein that has at least one DNA binding domain that binds to DNA binding sites present in the promoter regions of genes. Thus, for gene expression to occur, a protein comprising a DNA binding domain and a transactivation  
25 domain located at an appropriate distance from the DNA binding domain must be brought into the correct position in the promoter region of the gene.

The traditional transgenic approach utilizes a cell-type specific promoter to drive the expression of a designed transgene. A DNA construct containing the transgene is first incorporated into a host genome. When triggered by a transcriptional activator, expression of the transgene occurs in a given cell  
30 type.

Another means to regulate expression of foreign genes in cells is through inducible promoters. Examples of the use of such inducible promoters include the PR1-a promoter, prokaryotic repressor-operator systems, immunosuppressive-immunophilin systems, and higher eukaryotic transcription activation systems such as steroid hormone receptor systems and are described below.

35 The PR1-a promoter from tobacco is induced during the systemic acquired resistance response following pathogen attack. The use of PR1-a may be limited because it often responds to endogenous materials and external factors such as pathogens, UV-B radiation, and pollutants. Gene regulation systems based on promoters induced by heat shock, interferon and heavy metals have been described

(Wurn et al., 1986, Proc. Natl. Acad. Sci. USA 83: 5414-5418; Arnheiter et al., 1990, Cell 62: 51-61; Filmus et al., 1992, Nucleic Acids Research 20: 27550-27560). However, these systems have limitations due to their effect on expression of non-target genes. These systems are also leaky.

Prokaryotic repressor-operator systems utilize bacterial repressor proteins and the unique  
5 operator DNA sequences to which they bind. Both the tetracycline ("Tet") and lactose ("Lac")  
repressor-operator systems from the bacterium *Escherichia coli* have been used in plants and animals to  
control gene expression. In the Tet system, tetracycline binds to the TetR repressor protein, resulting in  
a conformational change which releases the repressor protein from the operator which as a result allows  
transcription to occur. In the Lac system, a lac operon is activated in response to the presence of lactose,  
10 or synthetic analogs such as isopropyl-b-D-thiogalactoside. Unfortunately, the use of such systems is  
restricted by unstable chemistry of the ligands, *i.e.* tetracycline and lactose, their toxicity, their natural  
presence, or the relatively high levels required for induction or repression. For similar reasons, utility of  
such systems in animals is limited.

Immunosuppressive molecules such as FK506, rapamycin and cyclosporine A can bind to  
15 immunophilins FKBP12, cyclophilin, *etc.* Using this information, a general strategy has been devised to  
bring together any two proteins simply by placing FK506 on each of the two proteins or by placing  
FK506 on one and cyclosporine A on another one. A synthetic homodimer of FK506 (FK1012) or a  
compound resulted from fusion of FK506-cyclosporine (FKCsA) can then be used to induce dimerization  
of these molecules (Spencer et al., 1993, *Science* 262:1019-24; Belshaw et al., 1996, *Proc Natl Acad Sci*  
20 *USA* 93:4604-7). Gal4 DNA binding domain fused to FKBP12 and VP16 activator domain fused to  
cyclophilin, and FKCsA compound were used to show heterodimerization and activation of a reporter  
gene under the control of a promoter containing Gal4 binding sites. Unfortunately, this system includes  
immunosuppressants that can have unwanted side effects and therefore, limits its use for various  
mammalian gene switch applications.

25 Higher eukaryotic transcription activation systems such as steroid hormone receptor systems  
have also been employed. Steroid hormone receptors are members of the nuclear receptor superfamily  
and are found in vertebrate and invertebrate cells. Unfortunately, use of steroidal compounds that  
activate the receptors for the regulation of gene expression, particularly in plants and mammals, is  
limited due to their involvement in many other natural biological pathways in such organisms. In order  
30 to overcome such difficulties, an alternative system has been developed using insect ecdysone receptors  
(EcR).

Growth, molting, and development in insects are regulated by the ecdysone steroid hormone  
(molting hormone) and the juvenile hormones (Dhadialla, et al., 1998, *Annu. Rev. Entomol.* 43: 545-  
569). The molecular target for ecdysone in insects consists of at least ecdysone receptor (EcR) and  
35 ultraspiracle protein (USP). EcR is a member of the nuclear steroid receptor super family that is  
characterized by signature DNA and ligand binding domains, and an activation domain (Koelle et al.  
1991, *Cell*, 67:59-77). EcR receptors are responsive to a number of steroidal compounds such as

ponasterone A and muristerone A. Recently, non-steroidal compounds with ecdysteroid agonist activity have been described, including the commercially available insecticides tebufenozide and methoxyfenozide that are marketed world wide by Rohm and Haas Company (see International Patent Application No. PCT/EP96/00686 and US Patent 5,530,028). Both analogs have exceptional safety  
5 profiles to other organisms.

International Patent Applications No. PCT/US97/05330 (WO 97/38117) and PCT/US99/08381 (WO99/58155) disclose methods for modulating the expression of an exogenous gene in which a DNA construct comprising the exogenous gene and an ecdysone response element is activated by a second DNA construct comprising an ecdysone receptor that, in the presence of a ligand therefor, and optionally  
10 in the presence of a receptor capable of acting as a silent partner, binds to the ecdysone response element to induce gene expression. The ecdysone receptor of choice was isolated from *Drosophila melanogaster*. Typically, such systems require the presence of the silent partner, preferably retinoid X receptor (RXR), in order to provide optimum activation. In mammalian cells, insect ecdysone receptor (EcR) heterodimerizes with retinoid X receptor (RXR) and regulates expression of target genes in a ligand  
15 dependent manner. International Patent Application No. PCT/US98/14215 (WO 99/02683) discloses that the ecdysone receptor isolated from the silk moth *Bombyx mori* is functional in mammalian systems without the need for an exogenous dimer partner.

U.S. Patent No. 5,880,333 discloses a *Drosophila melanogaster* EcR and ultraspiracle (USP) heterodimer system used in plants in which the transactivation domain and the DNA binding domain are  
20 positioned on two different hybrid proteins. Unfortunately, this system is not effective for inducing reporter gene expression in animal cells (for comparison, see Example 1.2, below).

In each of these cases, the transactivation domain and the DNA binding domain (either as native EcR as in International Patent Application No. PCT/US98/14215 or as modified EcR as in International Patent Application No. PCT/US97/05330) were incorporated into a single molecule and the other  
25 heterodimeric partners, either USP or RXR, were used in their native state.

Drawbacks of the above described EcR-based gene regulation systems include a considerable background activity in the absence of ligands and non-applicability of these systems for use in both plants and animals (see U.S. Patent No. 5,880,333). For most applications that rely on modulating gene expression, these EcR-based systems are undesirable. Therefore, a need exists in the art for improved  
30 systems to precisely modulate the expression of exogenous genes in both plants and animals. Such improved systems would be useful for applications such as gene therapy, large-scale production of proteins and antibodies, cell-based high throughput screening assays, functional genomics and regulation of traits in transgenic animals. Improved systems that are simple, compact, and dependent on ligands that are relatively inexpensive, readily available, and of low toxicity to the host would prove useful for  
35 regulating biological systems.

Recently, Applicants have shown that an ecdysone receptor-based inducible gene expression system in which the transactivation and DNA binding domains are separated from each other by placing

them on two different proteins results in greatly reduced background activity in the absence of a ligand and significantly increased activity over background in the presence of a ligand (pending application PCT/US01/09050, incorporated herein in its entirety by reference). This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems  
5 disclosed in applications PCT/US97/05330 and PCT/US98/14215.

Applicants previously demonstrated that an ecdysone receptor-based gene expression system in partnership with a dipteran (*Drosophila melanogaster*) or a lepidopteran (*Choristoneura fumiferana*) ultraspiracle protein (USP) is constitutively expressed in mammalian cells, while an ecdysone receptor-based gene expression system in partnership with a vertebrate retinoid X receptor (RXR) is inducible in  
10 mammalian cells (pending application PCT/US01/09050). Applicants have recently made the surprising discovery that a non-Dipteran and non-Lepidopteran invertebrate RXR can function similar to vertebrate RXR in an ecdysone receptor-based inducible gene expression system (US application filed concurrently herewith).

Applicants have now shown that a chimeric RXR ligand binding domain, comprising at least two  
15 polypeptide fragments, wherein the first polypeptide fragment is from one species of vertebrate/invertebrate RXR and the second polypeptide fragment is from a different species of vertebrate/invertebrate RXR, whereby a vertebrate/invertebrate chimeric RXR ligand binding domain, a vertebrate/vertebrate chimeric RXR ligand binding domain, or an invertebrate/invertebrate chimeric RXR ligand binding domain is produced, can function similar to or better than either the parental  
20 vertebrate RXR or the parental invertebrate RXR in an ecdysone receptor-based inducible gene expression system. As described herein, Applicants' novel ecdysone receptor/chimeric retinoid X receptor-based inducible gene expression system provides an inducible gene expression system in bacteria, fungi, yeast, animal, and mammalian cells that is characterized by increased ligand sensitivity and magnitude of transactivation.

25

### **SUMMARY OF THE INVENTION**

The present invention relates to a novel ecdysone receptor/chimeric retinoid X receptor-based inducible gene expression system, novel chimeric receptor polynucleotides and polypeptides for use in  
30 the novel inducible gene expression system, and methods of modulating the expression of a gene within a host cell using this inducible gene expression system. In particular, Applicants' invention relates to a novel gene expression modulation system comprising a polynucleotide encoding a chimeric RXR ligand binding domain (LBD).

Specifically, the present invention relates to a gene expression modulation system comprising: a)  
35 a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising: i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an

ecdysone receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising: i) a transactivation domain; and ii) a chimeric retinoid X receptor ligand binding domain.

5       The present invention also relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising: i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) a chimeric retinoid X receptor ligand binding domain; and b) a second gene expression cassette that is capable of being  
10 expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising: i) a transactivation domain; and ii) an ecdysone receptor ligand binding domain.

      The present invention also relates to a gene expression modulation system according to the invention further comprising c) a third gene expression cassette comprising: i) a response element to which the DNA-binding domain of the first hybrid polypeptide binds; ii) a promoter that is activated by  
15 the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated.

      The present invention also relates to a gene expression cassette that is capable of being expressed in a host cell, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated  
20 with a gene whose expression is to be modulated, or ii) a transactivation domain; and a chimeric retinoid X receptor ligand binding domain.

      The present invention also relates to an isolated polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated, or ii) a transactivation domain; and a chimeric  
25 vertebrate and invertebrate retinoid X receptor ligand binding domain. The present invention also relates to a isolated hybrid polypeptide encoded by the isolated polynucleotide according to the invention.

      The present invention also relates to an isolated polynucleotide encoding a truncated chimeric RXR LBD. In a specific embodiment, the isolated polynucleotide encodes a truncated chimeric RXR LBD, wherein the truncation mutation affects ligand binding activity or ligand sensitivity of the chimeric  
30 RXR LBD. In another specific embodiment, the isolated polynucleotide encodes a truncated chimeric RXR polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the truncated chimeric RXR polypeptide and a dimerization partner. In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide.

      The present invention also relates to an isolated polypeptide encoded by a polynucleotide  
35 according to Applicants' invention.

      The present invention also relates to an isolated hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be

modulated, or ii) a transactivation domain; and a chimeric retinoid X receptor ligand binding domain.

The present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation, wherein the truncated chimeric RXR LBD is encoded by a polynucleotide according to the invention.

5 Thus, the present invention also relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that affects ligand binding activity or ligand sensitivity of said truncated chimeric RXR LBD.

The present invention also relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the truncated chimeric  
10 RXR LBD and a dimerization partner. In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide.

Applicants' invention also relates to methods of modulating gene expression in a host cell using a gene expression modulation system according to the invention. Specifically, Applicants' invention provides a method of modulating the expression of a gene in a host cell comprising the steps  
15 of: a) introducing into the host cell a gene expression modulation system according to the invention; b) introducing into the host cell a gene expression cassette comprising i) a response element comprising a domain recognized by the DNA binding domain from the first hybrid polypeptide; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated; and c) introducing into the host cell a ligand; whereby upon introduction  
20 of the ligand into the host, expression of the gene of b)iii) is modulated.

Applicants' invention also provides a method of modulating the expression of a gene in a host cell comprising a gene expression cassette comprising a response element comprising a domain recognized by the DNA binding domain from the first hybrid polypeptide; a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and a gene whose expression is to be  
25 modulated; wherein the method comprises the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; and b) introducing into the host cell a ligand; whereby upon introduction of the ligand into the host, expression of the gene is modulated.

Applicants' invention also provides an isolated host cell comprising an inducible gene expression system according to the invention. The present invention also relates to an isolated host cell  
30 comprising a gene expression cassette, a polynucleotide, or a polypeptide according to the invention. Accordingly, Applicants' invention also relates to a non-human organism comprising a host cell according to the invention.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

35

Figure 1: Expression data of VP16LmUSP-EF, VP16MmRXR $\alpha$ -EF and three independent clones of VP16MmRXR $\alpha$ (1-7)-LmUSP (8-12)-EF in NIH3T3 cells along with GAL4CfEcR-CDEF and pFRLuc in



the presence of non-steroid (GSE) ligand.

Figure 2: Expression data of VP16LmUSP-EF, VP16MmRXR $\alpha$ -EF and two independent clones of VP16MmRXR $\alpha$ (1-7)-LmUSP (8-12)-EF in NIH3T3 cells along with GAL4CfEcR-CDEF and pFRLuc in the presence of non-steroid (GSE) ligand.

- 5 Figure 3: Expression data of VP16LmUSP-EF, VP16MmRXR $\alpha$ -EF and two independent clones of VP16MmRXR $\alpha$ (1-7)-LmUSP (8-12)-EF in A549 cells along with GAL4CfEcR-CDEF and pFRLuc in the presence of non-steroid (GSE) ligand.

- Figure 4: Amino acid sequence alignments of the EF domains of six vertebrate RXRs (A) and six invertebrate RXRs (B). B6, B8, B9, B10 and B11 denotes  $\beta$ chimera junctions. A1 denotes junction for  
10  $\alpha$ chimera. Helices 1-12 are denoted as H1-H12 and  $\beta$  pleated sheets are denoted as S1 and S2. F denotes the F domain junction.

Figure 5: Expression data of GAL4CfEcR-CDEF/VP16chimeric RXR-based gene switches 1.3-1.6 in NIH3T3 cells along with pFRLuc in the presence of non-steroid (GSE) ligand.

- Figure 6: Expression data of gene switches comprising the DEF domains of EcRs from CfEcR, DmEcR, TmEcR, or AmaEcR fused to GAL4 DNA binding domain and the EF domains of RXR/USPs from CfUSP, DmUSP, LmUSP, MmRXR $\alpha$ , a chimera between MmRXR $\alpha$  and LmUSP (Chimera), AmaRXR1, or AmaRXR2 fused to a VP16 activation domain along with pFRLuc in NIH3T3 cells in the presence of steroid (PonA) or non-steroid (GSE) ligand. The different RXR/USP constructs were compared in partnership with GAL4CfEcR-DEF.  
15

- Figure 7: Expression data of gene switches comprising the DEF domains of EcRs from CfEcR, DmEcR, TmEcR, or AmaEcR fused to GAL4 DNA binding domain and the EF domains of RXR/USPs from CfUSP, DmUSP, LmUSP, MmRXR $\alpha$ , a chimera between MmRXR $\alpha$  and LmUSP (Chimera), AmaRXR1, or AmaRXR2 fused to a VP16 activation domain along with pFRLuc in NIH3T3 cells in the presence of steroid (PonA) or non-steroid (GSE) ligand. The different RXR/USP constructs were compared in partnership with GAL4DmEcR-DEF.  
20  
25

- Figure 8: Expression data of gene switches comprising the DEF domains of EcRs from CfEcR, DmEcR, TmEcR, or AmaEcR fused to GAL4 DNA binding domain and the EF domains of RXR/USPs from CfUSP, DmUSP, LmUSP, MmRXR $\alpha$ , a chimera between MmRXR $\alpha$  and LmUSP (Chimera), AmaRXR1, or AmaRXR2 fused to a VP16 activation domain along with pFRLuc in NIH3T3 cells in the presence of steroid (PonA) or non-steroid (GSE) ligand. The different EcR constructs were compared in partnership with a chimeric RXR-EF (MmRXR $\alpha$ -(1-7)-LmUSP(8-12)-EF).  
30

- Figure 9: Expression data of VP16/MmRXR $\alpha$ -EF (aRXR), VP16/Chimera between MmRXR $\alpha$ -EF and LmUSP-EF (MmRXR $\alpha$ -(1-7)-LmUSP(8-12)-EF; aCh7), VP16/LmUSP-EF (LmUSP) and three independent clones from each of five VP16/chimeras between HsRXR $\beta$ -EF and LmUSP-EF (see Table 1 for chimeric RXR constructs; bRXRCh6, bRXRCh8, bRXRCh9, bRXRCh10, and bRXRCh11) were transfected into NIH3T3 cells along with GAL4/CfEcR-DEF and pFRLuc. The transfected cells were  
35

grown in the presence of 0, 0.2, 1 and 10  $\mu$ M non-steroidal ligand (GSE). The reporter activity was quantified 48 hours after adding ligands.

Figure 10: Expression data of VP16/MmRXR $\alpha$ -EF (aRXR), VP16/Chimera between MmRXR $\alpha$ -EF and LmUSP-EF (MmRXR $\alpha$ -(1-7)-LmUSP(8-12)-EF; aCh7), VP16/LmUSP-EF (LmUSP) and three

5 independent clones from each of five VP16/chimeras between HsRXR $\beta$ -EF and LmUSP-EF (see Table 1 for chimeric RXR constructs; bRXRCh6, bRXRCh8, bRXRCh9, bRXRCh10, and bRXRCh11) were transfected into NIH3T3 cells along with GAL4/CfEcR-DEF and pFRLuc. The transfected cells were grown in the presence of 0, 0.2, 1 and 10  $\mu$ M steroid ligand (PonA) or 0, 0.04, 0.2, 1, and 10  $\mu$ M non-steroidal ligand (GSE). The reporter activity was quantified 48 hours after adding ligands.

10 Figure 11: Expression data of VP16/MmRXR $\alpha$ -EF (aRXR), VP16/Chimera between MmRXR $\alpha$ -EF and LmUSP-EF (MmRXR $\alpha$ -(1-7)-LmUSP(8-12)-EF; aCh7), VP16/LmUSP-EF (LmUSP) and three independent clones from each of five VP16/chimeras between HsRXR $\beta$ -EF and LmUSP-EF (see Table 1 for chimeric RXR constructs; bRXRCh6, bRXRCh8, bRXRCh9, bRXRCh10, and bRXRCh11) were transfected into NIH3T3 cells along with GAL4/DmEcR-DEF and pFRLuc. The transfected cells were  
15 grown in the presence of 0, 0.2, 1 and 10  $\mu$ M steroid ligand (PonA) or 0, 0.04, 0.2, 1, and 10  $\mu$ M non-steroidal ligand (GSE). The reporter activity was quantified 48 hours after adding ligands.

Figure 12: Effect of 9-cis-retinoic acid on transactivation potential of the GAL4CfEcR-DEF/VP16HsRXR $\beta$ -(1-8)-LmUSP-(9-12)-EF ( $\beta$ chimera 9) gene switch along with pFRLuc in NIH 3T3 cells in the presence of non-steroid (GSE) and 9-cis-retinoic acid (9Cis) for 48 hours.

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### **DETAILED DESCRIPTION OF THE INVENTION**

Applicants have now shown that chimeric RXR ligand binding domains are functional within an EcR-based inducible gene expression modulation system in mammalian cells and that these chimeric  
25 RXR LBDs exhibit advantageous ligand sensitivities and transactivation abilities. Thus, Applicants' invention provides a novel ecdysone receptor-based inducible gene expression system comprising a chimeric retinoid X receptor ligand binding domain that is useful for modulating expression of a gene of interest in a host cell. In a particularly desirable embodiment, Applicants' invention provides an inducible gene expression system that has a reduced level of background gene expression and responds  
30 to submicromolar concentrations of non-steroidal ligand. Thus, Applicants' novel inducible gene expression system and its use in methods of modulating gene expression in a host cell overcome the limitations of currently available inducible expression systems and provide the skilled artisan with an effective means to control gene expression.

The present invention is useful for applications such as gene therapy, large scale production of  
35 proteins and antibodies, cell-based high throughput screening assays, functional genomics, proteomics, and metabolomics analyses and regulation of traits in transgenic organisms, where control of gene

expression levels is desirable. An advantage of Applicants' invention is that it provides a means to regulate gene expression and to tailor expression levels to suit the user's requirements.

#### DEFINITIONS

5 In this disclosure, a number of terms and abbreviations are used. The following definitions are provided and should be helpful in understanding the scope and practice of the present invention.

In a specific embodiment, the term "about" or "approximately" means within 20%, preferably within 10%, more preferably within 5%, and even more preferably within 1% of a given value or range.

The term "substantially free" means that a composition comprising "A" (where "A" is a single  
10 protein, DNA molecule, vector, recombinant host cell, etc.) is substantially free of "B" (where "B" comprises one or more contaminating proteins, DNA molecules, vectors, etc.) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A". Preferably, "A" comprises at least about 90% by weight of the A + B species in the composition, most preferably at least about 99% by weight. It is also preferred that a composition,  
15 which is substantially free of contamination, contain only a single molecular weight species having the activity or characteristic of the species of interest.

The term "isolated" for the purposes of the present invention designates a biological material (nucleic acid or protein) that has been removed from its original environment (the environment in which it is naturally present). For example, a polynucleotide present in the natural state in a plant or an animal  
20 is not isolated, however the same polynucleotide separated from the adjacent nucleic acids in which it is naturally present, is considered "isolated". The term "purified" does not require the material to be present in a form exhibiting absolute purity, exclusive of the presence of other compounds. It is rather a relative definition.

A polynucleotide is in the "purified" state after purification of the starting material or of the  
25 natural material by at least one order of magnitude, preferably 2 or 3 and preferably 4 or 5 orders of magnitude.

A "nucleic acid" is a polymeric compound comprised of covalently linked subunits called nucleotides. Nucleic acid includes polyribonucleic acid (RNA) and polydeoxyribonucleic acid (DNA), both of which may be single-stranded or double-stranded. DNA includes but is not limited to cDNA,  
30 genomic DNA, plasmids DNA, synthetic DNA, and semi-synthetic DNA. DNA may be linear, circular, or supercoiled.

A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs  
35 thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary

structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in  
5 the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

The term "fragment" will be understood to mean a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide sequence  
10 identical to the reference nucleic acid. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. Such fragments comprise, or alternatively consist of, oligonucleotides ranging in length from at least 6, 8, 9, 10, 12, 15, 18, 20, 21, 22, 23, 24, 25, 30, 39, 40, 42, 45, 48, 50, 51, 54, 57, 60, 63, 66, 70, 75, 78, 80, 90, 100, 105, 120, 135, 150, 200, 300, 500, 720, 900, 1000 or 1500 consecutive nucleotides of a nucleic acid according  
15 to the invention.

As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

20 A "gene" refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA nucleic acids. "Gene" also refers to a nucleic acid fragment that expresses a specific protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene,  
25 comprising regulatory and/or coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. A chimeric gene may comprise coding sequences derived from different sources and/or regulatory sequences derived from different sources. "Endogenous gene"  
30 refers to a native gene in its natural location in the genome of an organism. A "foreign" gene or "heterologous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

35 "Heterologous" DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

The term "genome" includes chromosomal as well as mitochondrial, chloroplast and viral DNA

or RNA.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see 5 Sambrook *et al.*, 1989 *infra*). Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization.

- 10 Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a  $T_m$  of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate 15 stringency hybridization conditions correspond to a higher  $T_m$ , *e.g.*, 40% formamide, with 5x or 6x SCC. High stringency hybridization conditions correspond to the highest  $T_m$ , *e.g.*, 50% formamide, 5x or 6x SCC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible.

- The term "complementary" is used to describe the relationship between nucleotide bases that are 20 capable of hybridizing to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine. Accordingly, the instant invention also includes isolated nucleic acid fragments that are complementary to the complete sequences as disclosed or used herein as well as those substantially similar nucleic acid sequences.

- In a specific embodiment, the term "standard hybridization conditions" refers to a  $T_m$  of 55°C, 25 and utilizes conditions as set forth above. In a preferred embodiment, the  $T_m$  is 60°C; in a more preferred embodiment, the  $T_m$  is 65°C.

- Post-hybridization washes also determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 minutes (min), then repeated with 2X SSC, 0.5% SDS at 45°C for 30 minutes, and then repeated twice with 0.2X SSC, 0.5% 30 SDS at 50°C for 30 minutes. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C. Hybridization requires that the two nucleic acids comprise complementary sequences, although depending on the stringency of the hybridization, 35 mismatches between bases are possible.

The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of

similarity or homology between two nucleotide sequences, the greater the value of  $T_m$  for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher  $T_m$ ) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived (see Sambrook *et al.*, *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook *et al.*, *supra*, 11.7-11.8).

In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferable a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

The term "probe" refers to a single-stranded nucleic acid molecule that can base pair with a complementary single stranded target nucleic acid to form a double-stranded molecule. As used herein, the term "oligonucleotide" refers to a nucleic acid, generally of at least 18 nucleotides, that is hybridizable to a genomic DNA molecule, a cDNA molecule, a plasmid DNA or an mRNA molecule. Oligonucleotides can be labeled, *e.g.*, with  $^{32}\text{P}$ -nucleotides or nucleotides to which a label, such as biotin, has been covalently conjugated. A labeled oligonucleotide can be used as a probe to detect the presence of a nucleic acid. Oligonucleotides (one or both of which may be labeled) can be used as PCR primers, either for cloning full length or a fragment of a nucleic acid, or to detect the presence of a nucleic acid. An oligonucleotide can also be used to form a triple helix with a DNA molecule. Generally, oligonucleotides are prepared synthetically, preferably on a nucleic acid synthesizer. Accordingly, oligonucleotides can be prepared with non-naturally occurring phosphoester analog bonds, such as thioester bonds, etc.

A "primer" is an oligonucleotide that hybridizes to a target nucleic acid sequence to create a double stranded nucleic acid region that can serve as an initiation point for DNA synthesis under suitable conditions. Such primers may be used in a polymerase chain reaction.

"Polymerase chain reaction" is abbreviated PCR and means an *in vitro* method for enzymatically amplifying specific nucleic acid sequences. PCR involves a repetitive series of temperature cycles with each cycle comprising three stages: denaturation of the template nucleic acid to separate the strands of the target molecule, annealing a single stranded PCR oligonucleotide primer to the template nucleic acid, and extension of the annealed primer(s) by DNA polymerase. PCR provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

"Reverse transcription-polymerase chain reaction" is abbreviated RT-PCR and means an *in vitro* method for enzymatically producing a target cDNA molecule or molecules from an RNA molecule or molecules, followed by enzymatic amplification of a specific nucleic acid sequence or sequences within

the target cDNA molecule or molecules as described above. RT-PCR also provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

A DNA "coding sequence" is a double-stranded DNA sequence that is transcribed and translated  
5 into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. "Suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation  
10 recognition sequences, RNA processing site, effector binding site and stem-loop structure. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from mRNA, genomic DNA sequences, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation  
15 signal and transcription termination sequence will usually be located 3' to the coding sequence.

"Open reading frame" is abbreviated ORF and means a length of nucleic acid sequence, either DNA, cDNA or RNA, that comprises a translation start signal or initiation codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

The term "head-to-head" is used herein to describe the orientation of two polynucleotide  
20 sequences in relation to each other. Two polynucleotides are positioned in a head-to-head orientation when the 5' end of the coding strand of one polynucleotide is adjacent to the 5' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds away from the 5' end of the other polynucleotide. The term "head-to-head" may be abbreviated (5')-to-(5') and may also be indicated by the symbols ( $\leftarrow \rightarrow$ ) or ( $3' \leftarrow 5' 5' \rightarrow 3'$ ).

25 The term "tail-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a tail-to-tail orientation when the 3' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds toward the other polynucleotide. The term "tail-to-tail" may be abbreviated (3')-to-(3') and may also be indicated by the  
30 symbols ( $\rightarrow \leftarrow$ ) or ( $5' \rightarrow 3' 3' \leftarrow 5'$ ).

The term "head-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a head-to-tail orientation when the 5' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds in the  
35 same direction as that of the other polynucleotide. The term "head-to-tail" may be abbreviated (5')-to-(3') and may also be indicated by the symbols ( $\rightarrow \rightarrow$ ) or ( $5' \rightarrow 3' 5' \rightarrow 3'$ ).

The term "downstream" refers to a nucleotide sequence that is located 3' to reference nucleotide

sequence. In particular, downstream nucleotide sequences generally relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

The term "upstream" refers to a nucleotide sequence that is located 5' to reference nucleotide  
5 sequence. In particular, upstream nucleotide sequences generally relate to sequences that are located on the 5' side of a coding sequence or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

The terms "restriction endonuclease" and "restriction enzyme" refer to an enzyme that binds and cuts within a specific nucleotide sequence within double stranded DNA.

10 "Homologous recombination" refers to the insertion of a foreign DNA sequence into another DNA molecule, e.g., insertion of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of  
15 homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

Several methods known in the art may be used to propagate a polynucleotide according to the invention. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As described herein, the expression vectors which  
20 can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid and cosmid DNA vectors, to name but a few.

A "vector" is any means for the cloning of and/or transfer of a nucleic acid into a host cell. A vector may be a replicon to which another DNA segment may be attached so as to bring about the  
25 replication of the attached segment. A "replicon" is any genetic element (e.g., plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*, i.e., capable of replication under its own control. The term "vector" includes both viral and nonviral means for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors known in the art may be used to manipulate nucleic acids, incorporate response elements and promoters into genes,  
30 etc. Possible vectors include, for example, plasmids or modified viruses including, for example bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives, or the Bluescript vector. For example, the insertion of the DNA fragments corresponding to response elements and promoters into a suitable vector can be accomplished by ligating the appropriate DNA fragments into a chosen vector that has complementary cohesive termini. Alternatively, the ends of the  
35 DNA molecules may be enzymatically modified or any site may be produced by ligating nucleotide sequences (linkers) into the DNA termini. Such vectors may be engineered to contain selectable marker genes that provide for the selection of cells that have incorporated the marker into the cellular genome.



Such markers allow identification and/or selection of host cells that incorporate and express the proteins encoded by the marker.

Viral vectors, and particularly retroviral vectors, have been used in a wide variety of gene delivery applications in cells, as well as living animal subjects. Viral vectors that can be used include but are not limited to retrovirus, adeno-associated virus, pox, baculovirus, vaccinia, herpes simplex, Epstein-Barr, adenovirus, geminivirus, and caulimovirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers. In addition to a nucleic acid, a vector may also comprise one or more regulatory regions, and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (transfer to which tissues, duration of expression, etc.).

The term "plasmid" refers to an extra chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

A "cloning vector" is a "replicon", which is a unit length of a nucleic acid, preferably DNA, that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. Cloning vectors may be capable of replication in one cell type and expression in another ("shuttle vector").

Vectors may be introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267: 963-967; Wu and Wu, 1988, J. Biol. Chem. 263: 14621-14624; and Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

A polynucleotide according to the invention can also be introduced *in vivo* by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids *in vitro*. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., 1987, Proc. Natl. Acad. Sci. U.S.A. 84: 7413; Mackey, et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85: 8027-8031; and Ulmer et al., 1993, Science 259: 1745-1748). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, 1989, Science 337: 387-388). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in International Patent Publications WO95/18863 and WO96/17823, and in U.S. Patent No. 5,459,127. The use of lipofection to introduce exogenous genes into

the specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly preferred in a tissue with cellular heterogeneity, such as pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting (Mackey, et al., 1988, *supra*). Targeted peptides, *e.g.*, hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptide (*e.g.*, WO95/21931), peptides derived from DNA binding proteins (*e.g.*, WO96/25508), or a cationic polymer (*e.g.*, WO95/21931).

10 It is also possible to introduce a vector *in vivo* as a naked DNA plasmid (see U.S. Patents 5,693,622, 5,589,466 and 5,580,859). Receptor-mediated DNA delivery approaches can also be used (Curiel et al., 1992, Hum. Gene Ther. 3: 147-154; and Wu and Wu, 1987, J. Biol. Chem. 262: 4429-4432).

The term "transfection" means the uptake of exogenous or heterologous RNA or DNA by a cell.  
15 A cell has been "transfected" by exogenous or heterologous RNA or DNA when such RNA or DNA has been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous RNA or DNA when the transfected RNA or DNA effects a phenotypic change. The transforming RNA or DNA can be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host  
20 organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

The term "genetic region" will refer to a region of a nucleic acid molecule or a nucleotide sequence that comprises a gene encoding a polypeptide.

In addition, the recombinant vector comprising a polynucleotide according to the invention may  
25 include one or more origins for replication in the cellular hosts in which their amplification or their expression is sought, markers or selectable markers.

The term "selectable marker" means an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, *i.e.*, resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like,  
30 wherein the effect is used to track the inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like.

35 The term "reporter gene" means a nucleic acid encoding an identifying factor that is able to be identified based upon the reporter gene's effect, wherein the effect is used to track the inheritance of a

nucleic acid of interest, to identify a cell or organism that has inherited the nucleic acid of interest, and/or to measure gene expression induction or transcription. Examples of reporter genes known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT),  $\beta$ -galactosidase (LacZ),  $\beta$ -glucuronidase (Gus), and the like. Selectable marker  
5 genes may also be considered reporter genes.

"Promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by  
10 those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". Promoters that cause a gene to be expressed in a specific cell type are commonly referred to as "cell-specific promoters" or "tissue-specific promoters".  
15 Promoters that cause a gene to be expressed at a specific stage of development or cell differentiation are commonly referred to as "developmentally-specific promoters" or "cell differentiation-specific promoters". Promoters that are induced and cause a gene to be expressed following exposure or treatment of the cell with an agent, biological molecule, chemical, ligand, light, or the like that induces the promoter are commonly referred to as "inducible promoters" or "regulatable promoters". It is further  
20 recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site  
25 and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

A coding sequence is "under the control" of transcriptional and translational control sequences in  
30 a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced (if the coding sequence contains introns) and translated into the protein encoded by the coding sequence.

"Transcriptional and translational control sequences" are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a  
35 host cell. In eukaryotic cells, polyadenylation signals are control sequences.

The term "response element" means one or more cis-acting DNA elements which confer responsiveness on a promoter mediated through interaction with the DNA-binding domains of the first

chimeric gene. This DNA element may be either palindromic (perfect or imperfect) in its sequence or composed of sequence motifs or half sites separated by a variable number of nucleotides. The half sites can be similar or identical and arranged as either direct or inverted repeats or as a single half site or multimers of adjacent half sites in tandem. The response element may comprise a minimal promoter  
5 isolated from different organisms depending upon the nature of the cell or organism into which the response element will be incorporated. The DNA binding domain of the first hybrid protein binds, in the presence or absence of a ligand, to the DNA sequence of a response element to initiate or suppress transcription of downstream gene(s) under the regulation of this response element. Examples of DNA sequences for response elements of the natural ecdysone receptor include: RRGG/TTCANTGAC/ACYY  
10 (see Cherbas L., et. al., (1991), *Genes Dev.* 5, 120-131); AGGTCAN<sub>(n)</sub>AGGTCA, where N<sub>(n)</sub> can be one or more spacer nucleotides (see D'Avino PP., et. al., (1995), *Mol. Cell. Endocrinol.* 113, 1-9); and GGGTTGAATGAATTT (see Antoniewski C., et. al., (1994), *Mol. Cell Biol.* 14, 4465-4474).

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably  
15 linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from a nucleic acid or polynucleotide. Expression may also  
20 refer to translation of mRNA into a protein or polypeptide.

The terms "cassette", "expression cassette" and "gene expression cassette" refer to a segment of DNA that can be inserted into a nucleic acid or polynucleotide at specific restriction sites or by homologous recombination. The segment of DNA comprises a polynucleotide that encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the  
25 cassette in the proper reading frame for transcription and translation. "Transformation cassette" refers to a specific vector comprising a polynucleotide that encodes a polypeptide of interest and having elements in addition to the polynucleotide that facilitate transformation of a particular host cell. Cassettes, expression cassettes, gene expression cassettes and transformation cassettes of the invention may also comprise elements that allow for enhanced expression of a polynucleotide encoding a polypeptide of  
30 interest in a host cell. These elements may include, but are not limited to: a promoter, a minimal promoter, an enhancer, a response element, a terminator sequence, a polyadenylation sequence, and the like.

For purposes of this invention, the term "gene switch" refers to the combination of a response element associated with a promoter, and an EcR based system which, in the presence of one or more  
35 ligands, modulates the expression of a gene into which the response element and promoter are incorporated.

The terms "modulate" and "modulates" mean to induce, reduce or inhibit nucleic acid or gene

expression, resulting in the respective induction, reduction or inhibition of protein or polypeptide production.

The plasmids or vectors according to the invention may further comprise at least one promoter suitable for driving expression of a gene in a host cell. The term "expression vector" means a vector, 5 plasmid or vehicle designed to enable the expression of an inserted nucleic acid sequence following transformation into the host. The cloned gene, i.e., the inserted nucleic acid sequence, is usually placed under the control of control elements such as a promoter, a minimal promoter, an enhancer, or the like. Initiation control regions or promoters, which are useful to drive expression of a nucleic acid in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of 10 driving these genes is suitable for the present invention including but not limited to: viral promoters, bacterial promoters, animal promoters, mammalian promoters, synthetic promoters, constitutive promoters, tissue specific promoter, developmental specific promoters, inducible promoters, light regulated promoters; *CYC1*, *HIS3*, *GAL1*, *GAL4*, *GAL10*, *ADH1*, *PGK*, *PHOS*, *GAPDH*, *ADC1*, *TRP1*, *URA3*, *LEU2*, *ENO*, *TPI*, alkaline phosphatase promoters (useful for expression in *Saccharomyces*); 15 *AOX1* promoter (useful for expression in *Pichia*);  $\beta$ -lactamase, *lac*, *ara*, *tet*, *trp*, *lP<sub>L</sub>*, *lP<sub>R</sub>*, *T7*, *tac*, and *trc* promoters (useful for expression in *Escherichia coli*); light regulated-promoters; animal and mammalian promoters known in the art include, but are not limited to, the SV40 early (SV40e) promoter region, the promoter contained in the 3' long terminal repeat (LTR) of Rous sarcoma virus (RSV), the promoters of the E1A or major late promoter (MLP) genes of adenoviruses (Ad), the cytomegalovirus 20 (CMV) early promoter, the herpes simplex virus (HSV) thymidine kinase (TK) promoter, an elongation factor 1 alpha (EF1) promoter, a phosphoglycerate kinase (PGK) promoter, a ubiquitin (Ubc) promoter, an albumin promoter, the regulatory sequences of the mouse metallothionein-L promoter and transcriptional control regions, the ubiquitous promoters (HPRT, vimentin,  $\alpha$ -actin, tubulin and the like), the promoters of the intermediate filaments (desmin, neurofilaments, keratin, GFAP, and the like), the 25 promoters of therapeutic genes (of the MDR, CFTR or factor VIII type, and the like), pathogenesis or disease related-promoters, and promoters that exhibit tissue specificity and have been utilized in transgenic animals, such as the elastase I gene control region which is active in pancreatic acinar cells; insulin gene control region active in pancreatic beta cells, immunoglobulin gene control region active in lymphoid cells, mouse mammary tumor virus control region active in testicular, breast, lymphoid and 30 mast cells; albumin gene, Apo AI and Apo AII control regions active in liver, alpha-fetoprotein gene control region active in liver, alpha 1-antitrypsin gene control region active in the liver, beta-globin gene control region active in myeloid cells, myelin basic protein gene control region active in oligodendrocyte cells in the brain, myosin light chain-2 gene control region active in skeletal muscle, and gonadotropic releasing hormone gene control region active in the hypothalamus, pyruvate kinase promoter, villin 35 promoter, promoter of the fatty acid binding intestinal protein, promoter of the smooth muscle cell  $\alpha$ -actin, and the like. In addition, these expression sequences may be modified by addition of enhancer or regulatory sequences and the like.

Enhancers that may be used in embodiments of the invention include but are not limited to: an SV40 enhancer, a cytomegalovirus (CMV) enhancer, an elongation factor 1 (EF1) enhancer, yeast enhancers, viral gene enhancers, and the like.

Termination control regions, *i.e.*, terminator or polyadenylation sequences, may also be derived from various genes native to the preferred hosts. Optionally, a termination site may be unnecessary, however, it is most preferred if included. In a preferred embodiment of the invention, the termination control region may be comprised or be derived from a synthetic sequence, synthetic polyadenylation signal, an SV40 late polyadenylation signal, an SV40 polyadenylation signal, a bovine growth hormone (BGH) polyadenylation signal, viral terminator sequences, or the like.

10 The terms "3' non-coding sequences" or "3' untranslated region (UTR)" refer to DNA sequences located downstream (3') of a coding sequence and may comprise polyadenylation [poly(A)] recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor.

15 "Regulatory region" means a nucleic acid sequence which regulates the expression of a second nucleic acid sequence. A regulatory region may include sequences which are naturally responsible for expressing a particular nucleic acid (a homologous region) or may include sequences of a different origin that are responsible for expressing different proteins or even synthetic proteins (a heterologous region). In particular, the sequences can be sequences of prokaryotic, eukaryotic, or viral genes or derived sequences that stimulate or repress transcription of a gene in a specific or non-specific manner and in an inducible or non-inducible manner. Regulatory regions include origins of replication, RNA splice sites, promoters, enhancers, transcriptional termination sequences, and signal sequences which direct the polypeptide into the secretory pathways of the target cell.

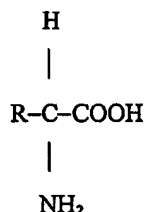
20 A regulatory region from a "heterologous source" is a regulatory region that is not naturally associated with the expressed nucleic acid. Included among the heterologous regulatory regions are regulatory regions from a different species, regulatory regions from a different gene, hybrid regulatory sequences, and regulatory sequences which do not occur in nature, but which are designed by one having ordinary skill in the art.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene. The complementarity of an

antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

A "polypeptide" is a polymeric compound comprised of covalently linked amino acid residues.

5 Amino acids have the following general structure:



10

Amino acids are classified into seven groups on the basis of the side chain R: (1) aliphatic side chains, (2) side chains containing a hydroxylic (OH) group, (3) side chains containing sulfur atoms, (4) side chains containing an acidic or amide group, (5) side chains containing a basic group, (6) side chains containing an aromatic ring, and (7) proline, an imino acid in which the side chain is fused to the amino

15 group. A polypeptide of the invention preferably comprises at least about 14 amino acids.

A "protein" is a polypeptide that performs a structural or functional role in a living cell.

An "isolated polypeptide" or "isolated protein" is a polypeptide or protein that is substantially free of those compounds that are normally associated therewith in its natural state (e.g., other proteins or polypeptides, nucleic acids, carbohydrates, lipids). "Isolated" is not meant to exclude artificial or  
20 synthetic mixtures with other compounds, or the presence of impurities which do not interfere with biological activity, and which may be present, for example, due to incomplete purification, addition of stabilizers, or compounding into a pharmaceutically acceptable preparation.

"Fragment" of a polypeptide according to the invention will be understood to mean a polypeptide whose amino acid sequence is shorter than that of the reference polypeptide and which comprises, over  
25 the entire portion with these reference polypeptides, an identical amino acid sequence. Such fragments may, where appropriate, be included in a larger polypeptide of which they are a part. Such fragments of a polypeptide according to the invention may have a length of at least 2, 3, 4, 5, 6, 8, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 30, 35, 40, 45, 50, 100, 200, 240, or 300 amino acids.

A "variant" of a polypeptide or protein is any analogue, fragment, derivative, or mutant which is  
30 derived from a polypeptide or protein and which retains at least one biological property of the polypeptide or protein. Different variants of the polypeptide or protein may exist in nature. These variants may be allelic variations characterized by differences in the nucleotide sequences of the structural gene coding for the protein, or may involve differential splicing or post-translational modification. The skilled artisan can produce variants having single or multiple amino acid  
35 substitutions, deletions, additions, or replacements. These variants may include, *inter alia*: (a) variants in which one or more amino acid residues are substituted with conservative or non-conservative amino acids, (b) variants in which one or more amino acids are added to the polypeptide or protein, (c) variants

in which one or more of the amino acids includes a substituent group, and (d) variants in which the polypeptide or protein is fused with another polypeptide such as serum albumin. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, etc.), chemical, and enzymatic techniques, are known to persons having ordinary skill in the art. A variant polypeptide  
5 preferably comprises at least about 14 amino acids.

A "heterologous protein" refers to a protein not naturally produced in the cell.

A "mature protein" refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present.  
10 Pre- and propeptides may be but are not limited to intracellular localization signals.

The term "signal peptide" refers to an amino terminal polypeptide preceding the secreted mature protein. The signal peptide is cleaved from and is therefore not present in the mature protein. Signal peptides have the function of directing and translocating secreted proteins across cell membranes. Signal peptide is also referred to as signal protein.

15 A "signal sequence" is included at the beginning of the coding sequence of a protein to be expressed on the surface of a cell. This sequence encodes a signal peptide, N-terminal to the mature polypeptide, that directs the host cell to translocate the polypeptide. The term "translocation signal sequence" is used herein to refer to this sort of signal sequence. Translocation signal sequences can be found associated with a variety of proteins native to eukaryotes and prokaryotes, and are often functional  
20 in both types of organisms.

The term "homology" refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known to the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence  
25 information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s) and size determination of the digested fragments.

As used herein, the term "homologous" in all its grammatical forms and spelling variations refers  
30 to the relationship between proteins that possess a "common evolutionary origin," including proteins from superfamilies (e.g., the immunoglobulin superfamily) and homologous proteins from different species (e.g., myosin light chain, etc.) (Reeck et al., 1987, Cell 50:667.). Such proteins (and their encoding genes) have sequence homology, as reflected by their high degree of sequence similarity. However, in common usage and in the instant application, the term "homologous," when modified with  
35 an adverb such as "highly," may refer to sequence similarity and not a common evolutionary origin.

Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of proteins that may or may not



share a common evolutionary origin (*see* Reeck et al., 1987, Cell 50:667).

In a specific embodiment, two DNA sequences are "substantially homologous" or "substantially similar" when at least about 50% (preferably at least about 75%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are  
5 substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook *et al.*, 1989, *supra*.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one  
10 or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the  
15 instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript. It is therefore understood that the invention encompasses more than the specific exemplary sequences. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Moreover, the skilled artisan recognizes that substantially similar sequences encompassed by this  
20 invention are also defined by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS), with the sequences exemplified herein. Substantially similar nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are at least 70% identical to the DNA sequence of the  
25 nucleic acid fragments reported herein. Preferred substantially nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are at least 80% identical to the DNA sequence of the nucleic acid fragments reported herein. More preferred nucleic acid fragments are at least 90% identical to the DNA sequence of the nucleic acid fragments reported herein. Even more preferred are nucleic acid fragments that are at least 95% identical to the DNA sequence of the nucleic  
30 acid fragments reported herein.

Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than about 40% of the amino acids are identical, or greater than 60% are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, *Version 7*,  
35 Madison, Wisconsin) pileup program.

The term "corresponding to" is used herein to refer to similar or homologous sequences, whether the exact position is identical or different from the molecule to which the similarity or homology is

measured. A nucleic acid or amino acid sequence alignment may include spaces. Thus, the term "corresponding to" refers to the sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215: 403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence.

The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in: *Computational Molecular Biology* (Lesk, A. M., ed.) Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects* (Smith, D. W., ed.) Academic Press, New York (1993); *Computer Analysis of Sequence Data, Part I* (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology* (von Heinje, G., ed.) Academic Press (1987); and *Sequence Analysis Primer* (Gribskov, M. and Devereux, J., eds.) Stockton Press, New York (1991). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences may be performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method may be selected: KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

The term "sequence analysis software" refers to any computer algorithm or software program

that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence analysis software will include but is not limited to the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI), BLASTP, BLASTN, BLASTX (Altschul et al., *J. Mol. Biol.* 215:403-410  
5 (1990), and DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715 USA). Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the "default values" of the program referenced, unless otherwise specified. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

10 "Synthetic genes" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments that are then enzymatically assembled to construct the entire gene. "Chemically synthesized", as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of DNA may be accomplished using well-established  
15 procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from  
20 the host cell where sequence information is available.

#### GENE EXPRESSION MODULATION SYSTEM OF THE INVENTION

Applicants have previously shown that separating the transactivation and DNA binding domains by placing them on two different proteins results in greatly reduced background activity in the absence of  
25 a ligand and significantly increased activity over background in the presence of a ligand (pending application PCT/US01/09050). This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems disclosed in International Patent Applications PCT/US97/05330 and PCT/US98/14215. The two-hybrid system exploits the ability of a pair of interacting proteins to bring the transcription activation domain into a more favorable position  
30 relative to the DNA binding domain such that when the DNA binding domain binds to the DNA binding site on the gene, the transactivation domain more effectively activates the promoter (see, for example, U.S. Patent No. 5,283,173). Briefly, the two-hybrid gene expression system comprises two gene expression cassettes; the first encoding a DNA binding domain fused to a nuclear receptor polypeptide, and the second encoding a transactivation domain fused to a different nuclear receptor polypeptide. In  
35 the presence of ligand, the interaction of the first polypeptide with the second polypeptide effectively tethers the DNA binding domain to the transactivation domain. Since the DNA binding and transactivation domains reside on two different molecules, the background activity in the absence of

ligand is greatly reduced.

The two-hybrid ecdysone receptor-based gene expression modulation system may be either heterodimeric and homodimeric. A functional EcR complex generally refers to a heterodimeric protein complex consisting of two members of the steroid receptor family, an ecdysone receptor protein obtained  
5 from various insects, and an ultraspiracle (USP) protein or the vertebrate homolog of USP, retinoid X receptor protein (see Yao, et al. (1993) Nature 366, 476-479; Yao, et al., (1992) Cell 71, 63-72). However, the complex may also be a homodimer as detailed below. The functional ecdysteroid receptor complex may also include additional protein(s) such as immunophilins. Additional members of the steroid receptor family of proteins, known as transcriptional factors (such as DHR38 or *betaFTZ-I*), may  
10 also be ligand dependent or independent partners for EcR, USP, and/or RXR. Additionally, other cofactors may be required such as proteins generally known as coactivators (also termed adapters or mediators). These proteins do not bind sequence-specifically to DNA and are not involved in basal transcription. They may exert their effect on transcription activation through various mechanisms, including stimulation of DNA-binding of activators, by affecting chromatin structure, or by mediating  
15 activator-initiation complex interactions. Examples of such coactivators include RIP140, TIF1, RAP46/Bag-1, ARA70, SRC-1/NCoA-1, TIF2/GRIP/NCoA-2, ACTR/AIB1/RAC3/pCIP as well as the promiscuous coactivator C response element B binding protein, CBP/p300 (for review see Glass et al., Curr. Opin. Cell Biol. 9: 222-232, 1997). Also, protein cofactors generally known as corepressors (also known as repressors, silencers, or silencing mediators) may be required to effectively inhibit  
20 transcriptional activation in the absence of ligand. These corepressors may interact with the unliganded ecdysone receptor to silence the activity at the response element. Current evidence suggests that the binding of ligand changes the conformation of the receptor, which results in release of the corepressor and recruitment of the above described coactivators, thereby abolishing their silencing activity. Examples of corepressors include N-CoR and SMRT (for review, see Horwitz et al. Mol Endocrinol. 10:  
25 1167-1177, 1996). These cofactors may either be endogenous within the cell or organism, or may be added exogenously as transgenes to be expressed in either a regulated or unregulated fashion. Homodimer complexes of the ecdysone receptor protein, USP, or RXR may also be functional under some circumstances.

The ecdysone receptor complex typically includes proteins which are members of the nuclear  
30 receptor superfamily wherein all members are generally characterized by the presence of an amino-terminal transactivation domain, a DNA binding domain ("DBD"), and a ligand binding domain ("LBD") separated from the DBD by a hinge region. As used herein, the term "DNA binding domain" comprises a minimal polypeptide sequence of a DNA binding protein, up to the entire length of a DNA binding protein, so long as the DNA binding domain functions to associate with a particular response  
35 element. Members of the nuclear receptor superfamily are also characterized by the presence of four or five domains: A/B, C, D, E, and in some members F (see US patent 4,981,784 and Evans, Science 240:889-895 (1988)). The "A/B" domain corresponds to the transactivation domain, "C" corresponds to

the DNA binding domain, "D" corresponds to the hinge region, and "E" corresponds to the ligand binding domain. Some members of the family may also have another transactivation domain on the carboxy-terminal side of the LBD corresponding to "F".

The DBD is characterized by the presence of two cysteine zinc fingers between which are two amino acid motifs, the P-box and the D-box, which confer specificity for ecdysone response elements. These domains may be either native, modified, or chimeras of different domains of heterologous receptor proteins. This EcR receptor, like a subset of the steroid receptor family, also possesses less well-defined regions responsible for heterodimerization properties. Because the domains of EcR, USP, and RXR are modular in nature, the LBD, DBD, and transactivation domains may be interchanged.

Gene switch systems are known that incorporate components from the ecdysone receptor complex. However, in these known systems, whenever EcR is used it is associated with native or modified DNA binding domains and transactivation domains on the same molecule. USP or RXR are typically used as silent partners. Applicants have previously shown that when DNA binding domains and transactivation domains are on the same molecule the background activity in the absence of ligand is high and that such activity is dramatically reduced when DNA binding domains and transactivation domains are on different molecules, that is, on each of two partners of a heterodimeric or homodimeric complex (see PCT/US01/09050). This two-hybrid system also provides improved sensitivity to non-steroidal ligands for example, diacylhydrazines, when compared to steroidal ligands for example, ponasterone A ("PonA") or muristerone A ("MurA"). That is, when compared to steroids, the non-steroidal ligands provide higher activity at a lower concentration. In addition, since transactivation based on EcR gene switches is often cell-line dependent, it is easier to tailor switching systems to obtain maximum transactivation capability for each application. Furthermore, the two-hybrid system avoids some side effects due to overexpression of RXR that often occur when unmodified RXR is used as a switching partner. In a specific embodiment of the two-hybrid system, native DNA binding and transactivation domains of EcR or RXR are eliminated and as a result, these chimeric molecules have less chance of interacting with other steroid hormone receptors present in the cell resulting in reduced side effects.

Applicants have previously shown that an ecdysone receptor in partnership with a dipteran (fruit fly *Drosophila melanogaster*) or a lepidopteran (spruce bud worm *Choristoneura fumiferana*) ultraspiracle protein (USP) is constitutively expressed in mammalian cells, while an ecdysone receptor in partnership with a vertebrate retinoid X receptor (RXR) is inducible in mammalian cells (pending application PCT/US01/09050). Recently, Applicants made the surprising discovery that the ultraspiracle protein of *Locusta migratoria* ("LmUSP") and the RXR homolog 1 and RXR homolog 2 of the ixodid tick *Amblyomma americanum* ("AmaRXR1" and "AmaRXR2", respectively) and their non-Dipteran, non-Lepidopteran homologs including, but not limited to: fiddler crab *Celca pugilator* RXR homolog ("CpRXR"), beetle *Tenebrio molitor* RXR homolog ("TmRXR"), honeybee *Apis mellifera* RXR homolog ("AmRXR"), and an aphid *Myzus persicae* RXR homolog ("MpRXR"), all of which are

referred to herein collectively as invertebrate RXRs, can function similar to vertebrate retinoid X receptor (RXR) in an inducible ecdysone receptor-based inducible gene expression system in mammalian cells (US application filed herewith, incorporated by reference herein, in its entirety).

As described herein, Applicants have now discovered that a chimeric RXR ligand binding domain comprising at least two polypeptide fragments, wherein the first polypeptide fragment is from one species of vertebrate/invertebrate RXR and the second polypeptide fragment is from a different species of vertebrate/invertebrate RXR, whereby a vertebrate/invertebrate chimeric RXR ligand binding domain, a vertebrate/vertebrate chimeric RXR ligand binding domain, or an invertebrate/invertebrate chimeric RXR ligand binding domain is produced, can function in an ecdysone receptor-based inducible gene expression system. Surprisingly, Applicants' novel EcR/chimeric RXR-based inducible gene expression system can function similar to or better than both the EcR/vertebrate RXR-based gene expression system (PCT/US01/09050) and the EcR/invertebrate RXR-based gene expression system (US application filed herewith) in terms of ligand sensitivity and magnitude of gene induction. Thus, the present invention provides an improved EcR-based inducible gene expression system for use in bacterial, fungal, yeast, animal, and mammalian cells.

In particular, Applicants describe herein a novel two-hybrid system that comprises a chimeric RXR ligand binding domain. This novel gene expression system demonstrates for the first time that a polypeptide comprising a chimeric RXR ligand binding domain can function as a component of an inducible EcR-based inducible gene expression system in yeast and mammalian cells. As discussed herein, this finding is both unexpected and surprising.

Specifically, Applicants' invention relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an ecdysone receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell, wherein the second gene expression cassette comprises a polynucleotide sequence that encodes a second hybrid polypeptide comprising i) a transactivation domain; and ii) a chimeric retinoid X receptor ligand binding domain.

The present invention also relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) a chimeric retinoid X receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell, wherein the second gene expression cassette comprises a polynucleotide sequence that encodes a second hybrid polypeptide comprising i) a transactivation domain; and ii) an ecdysone receptor ligand binding domain.

The present invention also relates to a gene expression modulation system according to the

present invention further comprising c) a third gene expression cassette comprising: i) a response element to which the DNA-binding domain of the first hybrid polypeptide binds; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated.

- 5 In a specific embodiment, the gene whose expression is to be modulated is a homologous gene with respect to the host cell. In another specific embodiment, the gene whose expression is to be modulated is a heterologous gene with respect to the host cell.

The ligands for use in the present invention as described below, when combined with an EcR ligand binding domain and a chimeric RXR ligand binding domain, which in turn are bound to the response element linked to a gene, provide the means for external temporal regulation of expression of the gene. The binding mechanism or the order in which the various components of this invention bind to each other, that is, for example, ligand to receptor, first hybrid polypeptide to response element, second hybrid polypeptide to promoter, etc., is not critical. Binding of the ligand to the EcR ligand binding domain and the chimeric RXR ligand binding domain enables expression or suppression of the gene.

10 This mechanism does not exclude the potential for ligand binding to EcR or chimeric RXR, and the resulting formation of active homodimer complexes (e.g. EcR + EcR or chimeric RXR + chimeric RXR). Preferably, one or more of the receptor domains is varied producing a hybrid gene switch. Typically, one or more of the three domains, DBD, LBD, and transactivation domain, may be chosen from a source different than the source of the other domains so that the hybrid genes and the resulting hybrid proteins

20 are optimized in the chosen host cell or organism for transactivating activity, complementary binding of the ligand, and recognition of a specific response element. In addition, the response element itself can be modified or substituted with response elements for other DNA binding protein domains such as the GAL-4 protein from yeast (see Sadowski, et al. (1988), *Nature* 335: 563-564) or LexA protein from *Escherichia coli* (see Brent and Ptashne (1985), *Cell* 43: 729-736), or synthetic response elements

25 specific for targeted interactions with proteins designed, modified, and selected for such specific interactions (see, for example, Kim, et al. (1997), *Proc. Natl. Acad. Sci., USA* 94: 3616-3620) to accommodate hybrid receptors. Another advantage of two-hybrid systems is that they allow choice of a promoter used to drive the gene expression according to a desired end result. Such double control can be particularly important in areas of gene therapy, especially when cytotoxic proteins are produced, because

30 both the timing of expression as well as the cells wherein expression occurs can be controlled. When genes, operably linked to a suitable promoter, are introduced into the cells of the subject, expression of the exogenous genes is controlled by the presence of the system of this invention. Promoters may be constitutively or inducibly regulated or may be tissue-specific (that is, expressed only in a particular type of cells) or specific to certain developmental stages of the organism.

35

#### GENE EXPRESSION CASSETTES OF THE INVENTION

The novel EcR/chimeric RXR-based inducible gene expression system of the invention

comprises gene expression cassettes that are capable of being expressed in a host cell, wherein the gene expression cassettes each comprise a polynucleotide encoding a hybrid polypeptide. Thus, Applicants' invention also provides gene expression cassettes for use in the gene expression system of the invention.

5 Specifically, the present invention provides a gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide. In particular, the present invention provides a gene expression cassette that is capable of being expressed in a host cell, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element, or ii) a transactivation domain; and an ecdysone receptor  
10 ligand binding domain or a chimeric retinoid X receptor ligand binding domain.

In a specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain that recognizes a response element and an EcR ligand binding domain.

In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain that recognizes a response element and a chimeric RXR ligand  
15 binding domain.

In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain and an EcR ligand binding domain.

In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain and a chimeric RXR ligand binding domain.

20 In a preferred embodiment, the ligand binding domain (LBD) is an EcR LBD, a chimeric RXR LBD, or a related steroid/thyroid hormone nuclear receptor family member LBD or chimeric LBD, analog, combination, or modification thereof. In a specific embodiment, the LBD is an EcR LBD or a chimeric RXR LBD. In another specific embodiment, the LBD is from a truncated EcR LBD or a truncated chimeric RXR LBD. A truncation mutation may be made by any method used in the art,  
25 including but not limited to restriction endonuclease digestion/deletion, PCR-mediated/oligonucleotide-directed deletion, chemical mutagenesis, DNA strand breakage, and the like.

The EcR may be an invertebrate EcR, preferably selected from the class Arthropod. Preferably, the EcR is selected from the group consisting of a Lepidopteran EcR, a Dipteran EcR, an Orthopteran EcR, a Homopteran EcR and a Hemipteran EcR. More preferably, the EcR for use is a spruce budworm  
30 *Choristoneura fumiferana* EcR ("CfEcR"), a beetle *Tenebrio molitor* EcR ("TmEcR"), a *Manduca sexta* EcR ("MsEcR"), a *Heliothis virescens* EcR ("HvEcR"), a midge *Chironomus tentans* EcR ("CtEcR"), a silk moth *Bombyx mori* EcR ("BmEcR"), a fruit fly *Drosophila melanogaster* EcR ("DmEcR"), a mosquito *Aedes aegypti* EcR ("AaEcR"), a blowfly *Lucilia capitata* EcR ("LcEcR"), a blowfly *Lucilia cuprina* EcR ("LucEcR"), a Mediterranean fruit fly *Ceratitis capitata* EcR ("CcEcR"), a locust *Locusta migratoria* EcR ("LmEcR"), an aphid *Myzus persicae* EcR ("MpEcR"), a fiddler crab *Celula pugilator* EcR ("CpEcR"), an ixodid tick *Amblyomma americanum* EcR ("AmaEcR"), a whitefly *Bemisia argentifoli* EcR ("BaEcR", SEQ ID NO: 68) or a leafhopper *Nephotetix cincticeps* EcR ("NcEcR", SEQ



ID NO: 69). In a specific embodiment, the LBD is from spruce budworm (*Choristoneura fumiferana*) EcR ("CfEcR") or fruit fly *Drosophila melanogaster* EcR ("DmEcR").

In a specific embodiment, the EcR LBD comprises full-length EF domains. In a preferred embodiment, the full length EF domains are encoded by a polynucleotide comprising a nucleic acid  
5 sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

In a specific embodiment, the LBD is from a truncated EcR LBD. The EcR LBD truncation results in a deletion of at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, or 240 amino acids. In another specific embodiment, the  
10 EcR LBD truncation result in a deletion of at least a partial polypeptide domain. In another specific embodiment, the EcR LBD truncation results in a deletion of at least an entire polypeptide domain. More preferably, the EcR polypeptide truncation results in a deletion of at least an A/B-domain, a C-domain, a D-domain, an F-domain, an A/B/C-domains, an A/B/1/2-C-domains, an A/B/C/D-domains, an A/B/C/D/F-domains, an A/B/F-domains, an A/B/C/F-domains, a partial E-domain, or a partial F-domain.  
15 A combination of several partial and/or complete domain deletions may also be performed.

In one embodiment, the ecdysone receptor ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 (CfEcR-EF), SEQ ID NO: 2 (DmEcR-EF), SEQ ID NO: 3 (CfEcR-DE), and SEQ ID NO: 4 (DmEcR-DE).

In a preferred embodiment, the ecdysone receptor ligand binding domain is encoded by a  
20 polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF), SEQ ID NO: 67 (DmEcR-DEF), SEQ ID NO: 71 (TmEcR-DEF) and SEQ ID NO: 73 (AmaEcR-DEF).

In one embodiment, the ecdysone receptor ligand binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (CfEcR-EF), SEQ ID NO: 6 (DmEcR-EF),  
25 SEQ ID NO: 7 (CfEcR-DE), and SEQ ID NO: 8 (DmEcR-DE).

In a preferred embodiment, the ecdysone receptor ligand binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 72 (TmEcR-DEF), and SEQ ID NO: 74 (AmaEcR-DEF).

30 Preferably, the chimeric RXR ligand binding domain comprises at least two polypeptide fragments selected from the group consisting of a vertebrate species RXR polypeptide fragment, an invertebrate species RXR polypeptide fragment, and a non-Dipteran/non-Lepidopteran invertebrate species RXR homolog polypeptide fragment. A chimeric RXR ligand binding domain according to the invention may comprise at least two different species RXR polypeptide fragments, or when the species is  
35 the same, the two or more polypeptide fragments may be from two or more different isoforms of the species RXR polypeptide fragment.

In a specific embodiment, the vertebrate species RXR polypeptide fragment is from a mouse *Mus*

*musculus* RXR ("MmRXR") or a human *Homo sapiens* RXR ("HsRXR"). The RXR polypeptide may be an RXR<sub>α</sub>, RXR<sub>β</sub>, or RXR<sub>γ</sub> isoform.

In a preferred embodiment, the vertebrate species RXR polypeptide fragment is from a vertebrate species RXR-EF domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In another preferred embodiment, the vertebrate species RXR polypeptide fragment is from a vertebrate species RXR-EF domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

In another specific embodiment, the invertebrate species RXR polypeptide fragment is from a locust *Locusta migratoria* ultraspiracle polypeptide ("LmUSP"), an ixodid tick *Amblyomma americanum* RXR homolog 1 ("AmaRXR1"), a ixodid tick *Amblyomma americanum* RXR homolog 2 ("AmaRXR2"), a fiddler crab *Celca pugilator* RXR homolog ("CpRXR"), a beetle *Tenebrio molitor* RXR homolog ("TmRXR"), a honeybee *Apis mellifera* RXR homolog ("AmRXR"), and an aphid *Myzus persicae* RXR homolog ("MpRXR").

In a preferred embodiment, the invertebrate species RXR polypeptide fragment is from a invertebrate species RXR-EF domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26. In another preferred embodiment, the invertebrate species RXR polypeptide fragment is from a invertebrate species RXR-EF domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

In another specific embodiment, the invertebrate species RXR polypeptide fragment is from a non-Dipteran/non-Lepidopteran invertebrate species RXR homolog.

In a preferred embodiment, the chimeric RXR ligand binding domain comprises at least one vertebrate species RXR polypeptide fragment and one invertebrate species RXR polypeptide fragment.

In another preferred embodiment, the chimeric RXR ligand binding domain comprises at least one vertebrate species RXR polypeptide fragment and one non-Dipteran/non-Lepidopteran invertebrate species RXR homolog polypeptide fragment.

In another preferred embodiment, the chimeric RXR ligand binding domain comprises at least one invertebrate species RXR polypeptide fragment and one non-Dipteran/non-Lepidopteran invertebrate species RXR homolog polypeptide fragment.

In another preferred embodiment, the chimeric RXR ligand binding domain comprises at least one vertebrate species RXR polypeptide fragment and one different vertebrate species RXR polypeptide

fragment.

In another preferred embodiment, the chimeric RXR ligand binding domain comprises at least one invertebrate species RXR polypeptide fragment and one different invertebrate species RXR

polypeptide fragment.

In another preferred embodiment, the chimeric RXR ligand binding domain comprises at least one non-Dipteran/non-Lepidopteran invertebrate species RXR polypeptide fragment and one different non-Dipteran/non-Lepidopteran invertebrate species RXR polypeptide fragment.

5 In a specific embodiment, the chimeric RXR LBD comprises an RXR LBD domain comprising at least one polypeptide fragment selected from the group consisting of an EF-domain helix 1, an EF-domain helix 2, an EF-domain helix 3, an EF-domain helix 4, an EF-domain helix 5, an EF-domain helix 6, an EF-domain helix 7, an EF-domain helix 8, and EF-domain helix 9, an EF-domain helix 10, an EF-domain helix 11, an EF-domain helix 12, an F-domain, and an EF-domain  $\beta$ -pleated sheet, wherein the  
10 polypeptide fragment is from a different species RXR, i.e., chimeric to the RXR LBD domain, than the RXR LBD domain.

In another specific embodiment, the first polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 1-6, helices 1-7, helices 1-8, helices 1-9, helices 1-10, helices 1-11, or helices 1-12 of a first species RXR according to the invention, and the second polypeptide fragment of  
15 the chimeric RXR ligand binding domain comprises helices 7-12, helices 8-12, helices 9-12, helices 10-12, helices 11-12, helix 12, or F domain of a second species RXR according to the invention, respectively.

In a preferred embodiment, the first polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 1-6 of a first species RXR according to the invention, and the second  
20 polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 7-12 of a second species RXR according to the invention.

In another preferred embodiment, the first polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 1-7 of a first species RXR according to the invention, and the second polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 8-12 of a second  
25 species RXR according to the invention.

In another preferred embodiment, the first polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 1-8 of a first species RXR according to the invention, and the second polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 9-12 of a second species RXR according to the invention.

30 In another preferred embodiment, the first polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 1-9 of a first species RXR according to the invention, and the second polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 10-12 of a second species RXR according to the invention.

In another preferred embodiment, the first polypeptide fragment of the chimeric RXR ligand  
35 binding domain comprises helices 1-10 of a first species RXR according to the invention, and the second polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 11-12 of a second species RXR according to the invention.

In another preferred embodiment, the first polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 1-11 of a first species RXR according to the invention, and the second polypeptide fragment of the chimeric RXR ligand binding domain comprises helix 12 of a second species RXR according to the invention.

5 In another preferred embodiment, the first polypeptide fragment of the chimeric RXR ligand binding domain comprises helices 1-12 of a first species RXR according to the invention, and the second polypeptide fragment of the chimeric RXR ligand binding domain comprises an F domain of a second species RXR according to the invention.

In another specific embodiment, the LBD is from a truncated chimeric RXR ligand binding  
10 domain. The chimeric RXR LBD truncation results in a deletion of at least 1, 2, 3, 4, 5, 6, 8, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, or 240 amino acids. Preferably, the chimeric RXR LBD truncation results in a deletion of at least a partial polypeptide domain. More preferably, the chimeric RXR LBD truncation  
15 results in a deletion of at least an entire polypeptide domain. In a preferred embodiment, the chimeric RXR LBD truncation results in a deletion of at least a partial E-domain, a complete E-domain, a partial F-domain, a complete F-domain, an EF-domain helix 1, an EF-domain helix 2, an EF-domain helix 3, an EF-domain helix 4, an EF-domain helix 5, an EF-domain helix 6, an EF-domain helix 7, an EF-domain helix 8, and EF-domain helix 9, an EF-domain helix 10, an EF-domain helix 11, an EF-domain helix 12,  
20 or an EF-domain  $\beta$ -pleated sheet. A combination of several partial and/or complete domain deletions may also be performed.

In a preferred embodiment, the truncated chimeric RXR ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, or SEQ ID NO: 38. In another preferred embodiment, the truncated  
25 chimeric RXR ligand binding domain comprises a nucleic acid sequence of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, or SEQ ID NO: 44.

In a preferred embodiment, the chimeric RXR ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides  
30 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

35 In another preferred embodiment, the chimeric RXR ligand binding domain comprises an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino

acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

For purposes of this invention, EcR, vertebrate RXR, invertebrate RXR, and chimeric RXR also include synthetic and hybrid EcR, vertebrate RXR, invertebrate RXR, and chimeric RXR, and their homologs.

The DNA binding domain can be any DNA binding domain with a known response element, including synthetic and chimeric DNA binding domains, or analogs, combinations, or modifications thereof. Preferably, the DBD is a GAL4 DBD, a LexA DBD, a transcription factor DBD, a steroid/thyroid hormone nuclear receptor superfamily member DBD, a bacterial LacZ DBD, or a yeast put DBD. More preferably, the DBD is a GAL4 DBD [SEQ ID NO: 47 (polynucleotide) or SEQ ID NO: 48 (polypeptide)] or a LexA DBD [(SEQ ID NO: 49 (polynucleotide) or SEQ ID NO: 50 (polypeptide))].

The transactivation domain (abbreviated "AD" or "TA") may be any steroid/thyroid hormone nuclear receptor AD, synthetic or chimeric AD, polyglutamine AD, basic or acidic amino acid AD, a VP16 AD, a GAL4 AD, an NF- $\kappa$ B AD, a BP64 AD, a B42 acidic activation domain (B42AD), or an analog, combination, or modification thereof. In a specific embodiment, the AD is a synthetic or chimeric AD, or is obtained from a VP16, GAL4, NF- $\kappa$ B, or B42 acidic activation domain AD. Preferably, the AD is a VP16 AD [SEQ ID NO: 51 (polynucleotide) or SEQ ID NO: 52 (polypeptide)] or a B42 AD [SEQ ID NO: 53 (polynucleotide) or SEQ ID NO: 54 (polypeptide)].

In a preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 47) and a LexA DBD (SEQ ID NO: 49), and an EcR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF), SEQ ID NO: 67 (DmEcR-DEF), SEQ ID NO: 71 (TmEcR-DEF) and SEQ ID NO: 73 (AmaEcR-DEF).

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 48) and a LexA DBD (SEQ ID NO: 50), and an EcR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 72 (TmEcR-DEF), and SEQ ID NO: 74 (AmaEcR-DEF).

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 47) and a LexA DBD (SEQ ID NO: 49), and a chimeric RXR ligand binding domain encoded by a polynucleotide comprising a nucleic acid

sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) 5 nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group 10 consisting of a GAL4 DBD (SEQ ID NO: 48) and a LexA DBD (SEQ ID NO: 50), and a chimeric RXR ligand binding domain comprising an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 15 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence of 20 SEQ ID NO: 51 or SEQ ID NO: 53, and an EcR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF), SEQ ID NO: 67 (DmEcR-DEF), SEQ ID NO: 71 (TmEcR-DEF) and SEQ ID NO: 73 (AmaEcR-DEF).

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide 25 comprising a transactivation domain comprising an amino acid sequence of SEQ ID NO: 52 or SEQ ID NO: 54, and an EcR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 72 (TmEcR-DEF), and SEQ ID NO: 74 (AmaEcR-DEF).

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide 30 comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 51 or SEQ ID NO: 53 and a chimeric RXR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID 35 NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21,

and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence of SEQ ID NO: 52 or SEQ ID NO: 54 and a chimeric RXR ligand binding domain comprising an amino acid sequence selected from  
5 the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13  
10 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

The response element ("RE") may be any response element with a known DNA binding domain, or an analog, combination, or modification thereof. A single RE may be employed or multiple REs, either multiple copies of the same RE or two or more different REs, may be used in the present  
15 invention. In a specific embodiment, the RE is an RE from GAL4 ("GAL4RE"), LexA, a steroid/thyroid hormone nuclear receptor RE, or a synthetic RE that recognizes a synthetic DNA binding domain. Preferably, the RE is a GAL4RE comprising a polynucleotide sequence of SEQ ID NO: 55 or a LexARE (operon "op") comprising a polynucleotide sequence of SEQ ID NO: 56 (2XLexAop). Preferably, the first hybrid protein is substantially free of a transactivation domain and the second hybrid protein is  
20 substantially free of a DNA binding domain. For purposes of this invention, "substantially free" means that the protein in question does not contain a sufficient sequence of the domain in question to provide activation or binding activity.

Thus, the present invention also relates to a gene expression cassette comprising: i) a response element comprising a domain to which a polypeptide comprising a DNA binding domain binds; ii) a  
25 promoter that is activated by a polypeptide comprising a transactivation domain; and iii) a gene whose expression is to be modulated.

Genes of interest for use in Applicants' gene expression cassettes may be endogenous genes or heterologous genes. Nucleic acid or amino acid sequence information for a desired gene or protein can be located in one of many public access databases, for example, GENBANK, EMBL, Swiss-Prot, and  
30 PIR, or in many biology related journal publications. Thus, those skilled in the art have access to nucleic acid sequence information for virtually all known genes. Such information can then be used to construct the desired constructs for the insertion of the gene of interest within the gene expression cassettes used in Applicants' methods described herein.

Examples of genes of interest for use in Applicants' gene expression cassettes include, but are  
35 not limited to: genes encoding therapeutically desirable polypeptides or products that may be used to treat a condition, a disease, a disorder, a dysfunction, a genetic defect, such as monoclonal antibodies, enzymes, proteases, cytokines, interferons, insulin, erythropoietin, clotting factors, other blood factors or

components, viral vectors for gene therapy, virus for vaccines, targets for drug discovery, functional genomics, and proteomics analyses and applications, and the like.

#### POLYNUCLEOTIDES OF THE INVENTION

- 5           The novel ecdysone receptor/chimeric retinoid X receptor-based inducible gene expression system of the invention comprises a gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain, and b) an EcR ligand binding domain or a chimeric RXR ligand binding domain. These gene expression cassettes, the polynucleotides they comprise, and the hybrid polypeptides they encode are useful as components of an
- 10 EcR-based gene expression system to modulate the expression of a gene within a host cell.

Thus, the present invention provides an isolated polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain according to the invention, and b) an EcR ligand binding domain or a chimeric RXR ligand binding domain according to the invention.

- 15           The present invention also relates to an isolated polynucleotide that encodes a chimeric RXR ligand binding domain according to the invention.

- The present invention also relates to an isolated polynucleotide that encodes a truncated EcR LBD or a truncated chimeric RXR LBD comprising a truncation mutation according to the invention. Specifically, the present invention relates to an isolated polynucleotide encoding a truncated EcR or
- 20 chimeric RXR ligand binding domain comprising a truncation mutation that affects ligand binding activity or ligand sensitivity that is useful in modulating gene expression in a host cell.

- In a specific embodiment, the isolated polynucleotide encoding an EcR LBD comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF), SEQ ID NO: 67 (DmEcR-DEF), SEQ ID NO: 71 (TmEcR-DEF) and SEQ ID
- 25 NO: 73 (AmaEcR-DEF).

In another specific embodiment, the isolated polynucleotide encodes an EcR LBD comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 72 (TmEcR-DEF), and SEQ ID NO: 74 (AmaEcR-DEF).

- 30           In another specific embodiment, the isolated polynucleotide encoding a chimeric RXR LBD comprises a polynucleotide sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides
- 35 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.



In another specific embodiment, the isolated polynucleotide encodes a chimeric RXR LBD comprising an amino acid sequence consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of  
5 SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

In particular, the present invention relates to an isolated polynucleotide encoding a truncated  
10 chimeric RXR LBD comprising a truncation mutation, wherein the mutation reduces ligand binding activity or ligand sensitivity of the truncated chimeric RXR LBD. In a specific embodiment, the present invention relates to an isolated polynucleotide encoding a truncated chimeric RXR LBD comprising a truncation mutation that reduces steroid binding activity or steroid sensitivity of the truncated chimeric RXR LBD.

15 In another specific embodiment, the present invention relates to an isolated polynucleotide encoding a truncated chimeric RXR LBD comprising a truncation mutation that reduces non-steroid binding activity or non-steroid sensitivity of the truncated chimeric RXR LBD.

The present invention also relates to an isolated polynucleotide encoding a truncated chimeric RXR LBD comprising a truncation mutation, wherein the mutation enhances ligand binding activity or  
20 ligand sensitivity of the truncated chimeric RXR LBD. In a specific embodiment, the present invention relates to an isolated polynucleotide encoding a truncated chimeric RXR LBD comprising a truncation mutation that enhances steroid binding activity or steroid sensitivity of the truncated chimeric RXR LBD.

In another specific embodiment, the present invention relates to an isolated polynucleotide  
25 encoding a truncated chimeric RXR LBD comprising a truncation mutation that enhances non-steroid binding activity or non-steroid sensitivity of the truncated chimeric RXR LBD.

The present invention also relates to an isolated polynucleotide encoding a truncated chimeric retinoid X receptor LBD comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the truncated chimeric retinoid X receptor LBD and a dimerization partner. In a  
30 specific embodiment, the dimerization partner is an ecdysone receptor polypeptide. Preferably, the dimerization partner is a truncated EcR polypeptide. More preferably, the dimerization partner is an EcR polypeptide in which domains A/B have been deleted. Even more preferably, the dimerization partner is an EcR polypeptide comprising an amino acid sequence of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 72 (TmEcR-DEF) or SEQ ID NO: 74  
35 (AmaEcR-DEF).

### POLYPEPTIDES OF THE INVENTION

The novel ecdysone receptor/chimeric retinoid X receptor-based inducible gene expression system of the invention comprises a gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain, and b) an EcR ligand binding domain or a chimeric RXR ligand binding domain. These gene expression cassettes, the polynucleotides they comprise, and the hybrid polypeptides they encode are useful as components of an EcR/chimeric RXR-based gene expression system to modulate the expression of a gene within a host cell.

Thus, the present invention also relates to a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain according to the invention, and b) an EcR ligand binding domain or a chimeric RXR ligand binding domain according to the invention.

The present invention also relates to an isolated polypeptide comprising a chimeric RXR ligand binding domain according to the invention.

The present invention also relates to an isolated truncated EcR LBD or an isolated truncated chimeric RXR LBD comprising a truncation mutation according to the invention. Specifically, the present invention relates to an isolated truncated EcR LBD or an isolated truncated chimeric RXR LBD comprising a truncation mutation that affects ligand binding activity or ligand sensitivity.

In a specific embodiment, the isolated EcR LBD polypeptide is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF), SEQ ID NO: 67 (DmEcR-DEF), SEQ ID NO: 71 (TmEcR-DEF) and SEQ ID NO: 73 (AmaEcR-DEF).

In another specific embodiment, the isolated EcR LBD polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 72 (TmEcR-DEF), and SEQ ID NO: 74 (AmaEcR-DEF).

In another specific embodiment, the isolated truncated chimeric RXR LBD is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

In another specific embodiment, the isolated truncated chimeric RXR LBD comprises an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of

SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

5       The present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that reduces ligand binding activity or ligand sensitivity of the truncated chimeric RXR LBD.

      Thus, the present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that reduces ligand binding activity or ligand sensitivity of the truncated chimeric  
10 RXR LBD.

      In a specific embodiment, the present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that reduces steroid binding activity or steroid sensitivity of the truncated chimeric RXR LBD.

      In another specific embodiment, the present invention relates to an isolated truncated chimeric  
15 RXR LBD comprising a truncation mutation that reduces non-steroid binding activity or non-steroid sensitivity of the truncated chimeric RXR LBD.

      In addition, the present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that enhances ligand binding activity or ligand sensitivity of the truncated chimeric RXR LBD.

20       The present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that enhances ligand binding activity or ligand sensitivity of the truncated chimeric RXR LBD. In a specific embodiment, the present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that enhances steroid binding activity or steroid sensitivity of the truncated chimeric RXR LBD.

25       In another specific embodiment, the present invention relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that enhances non-steroid binding activity or non-steroid sensitivity of the truncated chimeric RXR LBD.

      The present invention also relates to an isolated truncated chimeric RXR LBD comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the truncated chimeric  
30 RXR LBD and a dimerization partner.

      In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide. Preferably, the dimerization partner is a truncated EcR polypeptide. Preferably, the dimerization partner is an EcR polypeptide in which domains A/B or A/B/C have been deleted. Even more preferably, the dimerization partner is an EcR polypeptide comprising an amino acid sequence of SEQ ID NO: 57  
35 (CfEcR-DEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 72 (TmEcR-DEF) or SEQ ID NO: 74 (AmaEcR-DEF).

### METHOD OF MODULATING GENE EXPRESSION OF THE INVENTION

Applicants' invention also relates to methods of modulating gene expression in a host cell using a gene expression modulation system according to the invention. Specifically, Applicants' invention provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; and b) introducing into the host cell a ligand; wherein the gene to be modulated is a component of a gene expression cassette comprising: i) a response element comprising a domain recognized by the DNA binding domain of the first hybrid polypeptide; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated, whereby upon introduction of the ligand into the host cell, expression of the gene is modulated.

The invention also provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; b) introducing into the host cell a gene expression cassette comprising i) a response element comprising a domain recognized by the DNA binding domain from the first hybrid polypeptide; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated; and c) introducing into the host cell a ligand; whereby expression of the gene is modulated in the host cell.

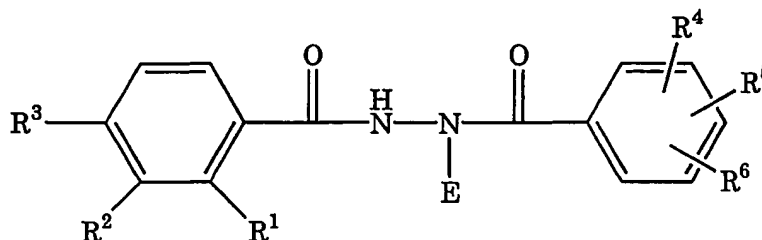
Genes of interest for expression in a host cell using Applicants' methods may be endogenous genes or heterologous genes. Nucleic acid or amino acid sequence information for a desired gene or protein can be located in one of many public access databases, for example, GENBANK, EMBL, Swiss-Prot, and PIR, or in many biology related journal publications. Thus, those skilled in the art have access to nucleic acid sequence information for virtually all known genes. Such information can then be used to construct the desired constructs for the insertion of the gene of interest within the gene expression cassettes used in Applicants' methods described herein.

Examples of genes of interest for expression in a host cell using Applicants' methods include, but are not limited to: genes encoding therapeutically desirable polypeptides or products that may be used to treat a condition, a disease, a disorder, a dysfunction, a genetic defect, such as monoclonal antibodies, enzymes, proteases, cytokines, interferons, insulin, erythropoietin, clotting factors, other blood factors or components, viral vectors for gene therapy, virus for vaccines, targets for drug discovery, functional genomics, and proteomics analyses and applications, and the like.

Acceptable ligands are any that modulate expression of the gene when binding of the DNA binding domain of the two-hybrid system to the response element in the presence of the ligand results in activation or suppression of expression of the genes. Preferred ligands include ponasterone, muristerone A, 9-cis-retinoic acid, synthetic analogs of retinoic acid, N,N'-diacylhydrazines such as those disclosed in U. S. Patents No. 6,013,836; 5,117,057; 5,530,028; and 5,378,726; dibenzoylalkyl cyanohydrazines such as those disclosed in European Application No. 461,809; N-alkyl-N,N'-diaroylhydrazines such as those disclosed in U. S. Patent No. 5,225,443; N-acyl-N-alkylcarbonylhydrazines such as those disclosed

in European Application No. 234,994; N-aryl-N-alkyl-N'-arylhydrazines such as those described in U. S. Patent No. 4,985,461; each of which is incorporated herein by reference and other similar materials including 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, and the like.

In a preferred embodiment, the ligand for use in Applicants' method of modulating expression of  
 5 gene is a compound of the formula:



wherein:

- E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;  
 R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN,  
 10 C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;  
 R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>,  
 15 OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;  
 R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a  
 20 dihydropyryl ring with the oxygen adjacent to a phenyl carbon;  
 R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

In another preferred embodiment, a second ligand may be used in addition to the first ligand discussed above in Applicants' method of modulating expression of a gene, wherein the second ligand is  
 25 9-cis-retinoic acid or a synthetic analog of retinoic acid.

Applicants' invention provides for modulation of gene expression in prokaryotic and eukaryotic host cells. Thus, the present invention also relates to a method for modulating gene expression in a host cell selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell. Preferably, the host cell is a yeast cell, a hamster cell, a mouse cell, a monkey cell, or a  
 30 human cell.

Expression in transgenic host cells may be useful for the expression of various polypeptides of interest including but not limited to therapeutic polypeptides, pathway intermediates; for the modulation of pathways already existing in the host for the synthesis of new products heretofore not possible using

the host; cell based assays; functional genomics assays, biotherapeutic protein production, proteomics assays, and the like. Additionally the gene products may be useful for conferring higher growth yields of the host or for enabling an alternative growth mode to be utilized.

## 5 HOST CELLS AND NON-HUMAN ORGANISMS OF THE INVENTION

As described above, the gene expression modulation system of the present invention may be used to modulate gene expression in a host cell. Expression in transgenic host cells may be useful for the expression of various genes of interest. Thus, Applicants' invention provides an isolated host cell comprising a gene expression system according to the invention. The present invention also provides an  
10 isolated host cell comprising a gene expression cassette according to the invention. Applicants' invention also provides an isolated host cell comprising a polynucleotide or a polypeptide according to the invention. The isolated host cell may be either a prokaryotic or a eukaryotic host cell.

Preferably, the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell. Examples of preferred host cells include, but are not  
15 limited to, fungal or yeast species such as *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, or bacterial species such as those in the genera *Synechocystis*, *Synechococcus*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Streptomyces*, *Escherichia*, *Pseudomonas*, *Methylobacter*, *Alcaligenes*, *Synechocystis*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and *Klebsiella*, animal, and mammalian host cells.

20 In a specific embodiment, the host cell is a yeast cell selected from the group consisting of a *Saccharomyces*, a *Pichia*, and a *Candida* host cell.

In another specific embodiment, the host cell is a hamster cell.

In another specific embodiment, the host cell is a murine cell.

In another specific embodiment, the host cell is a monkey cell.

25 In another specific embodiment, the host cell is a human cell.

Host cell transformation is well known in the art and may be achieved by a variety of methods including but not limited to electroporation, viral infection, plasmid/vector transfection, non-viral vector mediated transfection, particle bombardment, and the like. Expression of desired gene products involves culturing the transformed host cells under suitable conditions and inducing expression of the transformed  
30 gene. Culture conditions and gene expression protocols in prokaryotic and eukaryotic cells are well known in the art (see General Methods section of Examples). Cells may be harvested and the gene products isolated according to protocols specific for the gene product.

In addition, a host cell may be chosen which modulates the expression of the inserted polynucleotide, or modifies and processes the polypeptide product in the specific fashion desired.  
35 Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification [e.g., glycosylation, cleavage (e.g., of signal sequence)] of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and

processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce a non-glycosylated core protein product. However, a polypeptide expressed in bacteria may not be properly folded. Expression in yeast can produce a glycosylated product. Expression in eukaryotic cells can increase the likelihood of "native" glycosylation and folding of a heterologous protein.

- 5 Moreover, expression in mammalian cells can provide a tool for reconstituting, or constituting, the polypeptide's activity. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent.

Applicants' invention also relates to a non-human organism comprising an isolated host cell according to the invention. Preferably, the non-human organism is selected from the group consisting of  
10 a bacterium, a fungus, a yeast, an animal, and a mammal. More preferably, the non-human organism is a yeast, a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, or a chimpanzee.

In a specific embodiment, the non-human organism is a yeast selected from the group consisting of *Saccharomyces*, *Pichia*, and *Candida*.

- 15 In another specific embodiment, the non-human organism is a *Mus musculus* mouse.

#### MEASURING GENE EXPRESSION/TRANSCRIPTION

One useful measurement of Applicants' methods of the invention is that of the transcriptional state of the cell including the identities and abundances of RNA, preferably mRNA species. Such  
20 measurements are conveniently conducted by measuring cDNA abundances by any of several existing gene expression technologies.

Nucleic acid array technology is a useful technique for determining differential mRNA expression. Such technology includes, for example, oligonucleotide chips and DNA microarrays. These techniques rely on DNA fragments or oligonucleotides which correspond to different genes or cDNAs  
25 which are immobilized on a solid support and hybridized to probes prepared from total mRNA pools extracted from cells, tissues, or whole organisms and converted to cDNA. Oligonucleotide chips are arrays of oligonucleotides synthesized on a substrate using photolithographic techniques. Chips have been produced which can analyze for up to 1700 genes. DNA microarrays are arrays of DNA samples, typically PCR products, that are robotically printed onto a microscope slide. Each gene is analyzed by a  
30 full or partial-length target DNA sequence. Microarrays with up to 10,000 genes are now routinely prepared commercially. The primary difference between these two techniques is that oligonucleotide chips typically utilize 25-mer oligonucleotides which allow fractionation of short DNA molecules whereas the larger DNA targets of microarrays, approximately 1000 base pairs, may provide more sensitivity in fractionating complex DNA mixtures.

- 35 Another useful measurement of Applicants' methods of the invention is that of determining the translation state of the cell by measuring the abundances of the constituent protein species present in the cell using processes well known in the art.

Where identification of genes associated with various physiological functions is desired, an assay may be employed in which changes in such functions as cell growth, apoptosis, senescence, differentiation, adhesion, binding to a specific molecules, binding to another cell, cellular organization, organogenesis, intracellular transport, transport facilitation, energy conversion, metabolism, myogenesis, neurogenesis, and/or hematopoiesis is measured.

In addition, selectable marker or reporter gene expression may be used to measure gene expression modulation using Applicants' invention.

Other methods to detect the products of gene expression are well known in the art and include Southern blots (DNA detection), dot or slot blots (DNA, RNA), northern blots (RNA), RT-PCR (RNA), western blots (polypeptide detection), and ELISA (polypeptide) analyses. Although less preferred, labeled proteins can be used to detect a particular nucleic acid sequence to which it hybridizes.

In some cases it is necessary to amplify the amount of a nucleic acid sequence. This may be carried out using one or more of a number of suitable methods including, for example, polymerase chain reaction ("PCR"), ligase chain reaction ("LCR"), strand displacement amplification ("SDA"), transcription-based amplification, and the like. PCR is carried out in accordance with known techniques in which, for example, a nucleic acid sample is treated in the presence of a heat stable DNA polymerase, under hybridizing conditions, with one pair of oligonucleotide primers, with one primer hybridizing to one strand (template) of the specific sequence to be detected. The primers are sufficiently complementary to each template strand of the specific sequence to hybridize therewith. An extension product of each primer is synthesized and is complementary to the nucleic acid template strand to which it hybridized. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample may be analyzed as described above to assess whether the sequence or sequences to be detected are present.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention.

## EXAMPLES

### GENERAL METHODS

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y. (1989) (Maniatis) and by T. J. Silhavy, M. L. Bannan, and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) and by Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience (1987).

Materials and methods suitable for the maintenance and growth of bacterial cultures are well



known in the art. Techniques suitable for use in the following examples may be found as set out in *Manual of Methods for General Bacteriology* (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American Society for Microbiology, Washington, DC. (1994)) or by Thomas D. Brock in *Biotechnology: A Textbook of Industrial Microbiology*, Second Edition, Sinauer Associates, Inc., Sunderland, MA (1989). All reagents, restriction enzymes and materials used for the growth and maintenance of host cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

Manipulations of genetic sequences may be accomplished using the suite of programs available from the Genetics Computer Group Inc. (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI). Where the GCG program "Pileup" is used the gap creation default value of 12, and the gap extension default value of 4 may be used. Where the GCG "Gap" or "Bestfit" program is used the default gap creation penalty of 50 and the default gap extension penalty of 3 may be used. In any case where GCG program parameters are not prompted for, in these or any other GCG program, default values may be used.

The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "μl" means microliter(s), "ml" means milliliter(s), "L" means liter(s), "μM" means micromolar, "mM" means millimolar, "μg" means microgram(s), "mg" means milligram(s), "A" means adenine or adenosine, "T" means thymine or thymidine, "G" means guanine or guanosine, "C" means cytidine or cytosine, "x g" means times gravity, "nt" means nucleotide(s), "aa" means amino acid(s), "bp" means base pair(s), "kb" means kilobase(s), "k" means kilo, "μ" means micro, and "°C" means degrees Celsius.

### EXAMPLE 1

Applicants' EcR/chimeric RXR-based inducible gene expression modulation system is useful in various applications including gene therapy, expression of proteins of interest in host cells, production of transgenic organisms, and cell-based assays. Applicants have made the surprising discovery that a chimeric retinoid X receptor ligand binding domain can substitute for either parent RXR polypeptide and function inducibly in an EcR/chimeric RXR-based gene expression modulation system upon binding of ligand. In addition, the chimeric RXR polypeptide may also function better than either parent/donor RXR ligand binding domain. Applicants' surprising discovery and unexpected superior results provide a novel inducible gene expression system for bacterial, fungal, yeast, animal, and mammalian cell applications. This Example describes the construction of several gene expression cassettes for use in the EcR/chimeric RXR-based inducible gene expression system of the invention.

Applicants constructed several EcR-based gene expression cassettes based on the spruce budworm *Choristoneura fumiferana* EcR ("CfEcR"), *C. fumiferana* ultraspiracle ("CfUSP"), *Drosophila*

*melanogaster* EcR ("DmEcR"), *D. melanogaster* USP ("DmUSP"), *Tenebrio molitor* EcR ("TmEcR"), *Amblyomma americanum* EcR ("AmaEcR"), *A. americanum* RXR homolog 1 ("AmaRXR1"), *A. americanum* RXR homolog 2 ("AmaRXR2"), mouse *Mus musculus* retinoid X receptor  $\alpha$  isoform ("MmRXR $\alpha$ "), human *Homo sapiens* retinoid X receptor  $\beta$  isoform ("HsRXR $\beta$ "), and locust *Locusta migratoria* ultraspiracle ("LmUSP").

The prepared receptor constructs comprise 1) an EcR ligand binding domain (LBD), a vertebrate RXR (MmRXR $\alpha$  or HsRXR $\beta$ ) LBD, an invertebrate USP (CfUSP or DmUSP) LBD, an invertebrate RXR (LmUSP, AmaRXR1 or AmaRXR2) LBD, or a chimeric RXR LBD comprising a vertebrate RXR LBD fragment and an invertebrate RXR LBD fragment; and 2) a GAL4 or LexA DNA binding domain (DBD) or a VP16 or B42 acidic activator transactivation domain (AD). The reporter constructs include a reporter gene, luciferase or LacZ, operably linked to a synthetic promoter construct that comprises either a GAL4 response element or a LexA response element to which the Gal4 DBD or LexA DBD binds, respectively. Various combinations of these receptor and reporter constructs were cotransfected into mammalian cells as described in Examples 2-6 *infra*.

15

**Gene Expression Cassettes:** Ecdysone receptor-based gene expression cassette pairs (switches) were constructed as followed, using standard cloning methods available in the art. The following is brief description of preparation and composition of each switch used in the Examples described herein.

1.1 - GAL4CfEcR-CDEF/VP16MmRXR $\alpha$ -EF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-CDEF"; SEQ ID NO: 59) were fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 47) and placed under the control of an SV40e promoter (SEQ ID NO: 60). The EF domains from mouse *Mus musculus* RXR $\alpha$  ("MmRXR $\alpha$ -EF"; SEQ ID NO: 9) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 51) and placed under the control of an SV40e promoter (SEQ ID NO: 60). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 55) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 61) and placed upstream of the luciferase gene (SEQ ID NO: 62).

1.2 - Gal4CfEcR-CDEF/VP16LmUSP-EF: This construct was prepared in the same way as in switch 1.1 above except MmRXR $\alpha$ -EF was replaced with the EF domains from *Locusta migratoria* ultraspiracle ("LmUSP-EF"; SEQ ID NO: 21).

1.3 - Gal4CfEcR-CDEF/VP16MmRXR $\alpha$ (1-7)-LmUSP(8-12)-EF: This construct was prepared in the same way as in switch 1.1 above except MmRXR $\alpha$ -EF was replaced with helices 1 through 7 of MmRXR $\alpha$ -EF and helices 8 through 12 of LmUSP-EF (SEQ ID NO: 45).

1.4 - Gal4CfEcR-CDEF/VP16MmRXR $\alpha$ (1-7)-LmUSP(8-12)-EF-MmRXR $\alpha$ -F: This construct was prepared in the same way as in switch 1.1 above except MmRXR $\alpha$ -EF was replaced with helices 1 through 7 of MmRXR $\alpha$ -EF and helices 8 through 12 of LmUSP-EF (SEQ ID NO: 45), and wherein the last C-terminal 18 nucleotides of SEQ ID NO: 45 (F domain) were replaced with the F domain of

MmRXR $\alpha$  ("MmRXR $\alpha$ -F", SEQ ID NO: 63).

1.5 - Gal4CfEcR-CDEF/VP16MmRXR $\alpha$ (1-12)-EF-LmUSP-F: This construct was prepared in the same way as in switch 1.1 above except MmRXR $\alpha$ -EF was replaced with helices 1 through 12 of MmRXR $\alpha$ -EF (SEQ ID NO: 9) and wherein the last C-terminal 18 nucleotides of SEQ ID NO: 9 (F domain) were  
5 replaced with the F domain of LmUSP ("LmUSP-F", SEQ ID NO: 64).

1.6 - Gal4CfEcR-CDEF/VP16LmUSP(1-12)-EF-MmRXR $\alpha$ -F: This construct was prepared in the same way as in switch 1.1 above except MmRXR $\alpha$ -EF was replaced with helices 1 through 12 of LmUSP-EF (SEQ ID NO: 21) and wherein the last C-terminal 18 nucleotides of SEQ ID NO: 21 (F domain) were replaced with the F domain of MmRXR $\alpha$  ("MmRXR $\alpha$ -F", SEQ ID NO: 63).

10 1.7 - GAL4CfEcR-DEF/VP16CfUSP-EF: The D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-DEF", SEQ ID NO: 65) were fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD", SEQ ID NO: 47) and placed under the control of an SV40e promoter (SEQ ID NO: 60). The EF domains from *C. fumiferana* USP ("CfUSP-EF", SEQ ID NO: 66) were fused to the transactivation domain from VP16 ("VP16AD", SEQ ID NO: 51) and placed under the control of  
15 an SV40e promoter (SEQ ID NO: 60). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 55) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 61) and placed upstream of the luciferase gene (SEQ ID NO: 62).

1.8 - GAL4CfEcR-DEF/VP16DmUSP-EF: This construct was prepared in the same way as in switch 1.7  
20 above except CfUSP-EF was replaced with the corresponding EF domains from fruit fly *Drosophila melanogaster* USP ("DmUSP-EF", SEQ ID NO: 75).

1.9 - Gal4CfEcR-DEF/VP16LmUSP-EF: This construct was prepared in the same way as in switch 1.7 above except CfUSP-EF was replaced with the EF domains from *Locusta migratoria* USP ("LmUSP-EF", SEQ ID NO: 21).

25 1.10 - GAL4CfEcR-DEF/VP16MmRXR $\alpha$ -EF: This construct was prepared in the same way as in switch 1.7 above except CfUSP-EF was replaced with the EF domains of *M. musculus* MmRXR $\alpha$  ("MmRXR $\alpha$ -EF", SEQ ID NO: 9).

1.11 - GAL4CfEcR-DEF/VP16AmaRXR1-EF: This construct was prepared in the same way as in switch 1.7 above except CfUSP-EF was replaced with the EF domains of tick *Amblyomma americanum* RXR  
30 homolog 1 ("AmaRXR1-EF", SEQ ID NO: 22).

1.12 - GAL4CfEcR-DEF/VP16AmaRXR2-EF: This construct was prepared in the same way as in switch 1.7 above except CfUSP-EF was replaced with the EF domains of tick *A. americanum* RXR homolog 2 ("AmaRXR2-EF", SEQ ID NO: 23).

1.13 - Gal4CfEcR-DEF/VP16MmRXR $\alpha$ (1-7)-LmUSP(8-12)-EF (" $\alpha$ Chimera#7"): This construct was  
35 prepared in the same way as in switch 1.7 above except CfUSP-EF was replaced with helices 1 through 7 of MmRXR $\alpha$ -EF and helices 8 through 12 of LmUSP-EF (SEQ ID NO: 45).

1.14 - GAL4DmEcR-DEF/VP16CfUSP-EF: The D, E, and F domains from fruit fly *Drosophila*

*melanogaster* EcR ("DmEcR-DEF"; SEQ ID NO: 67) were fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 47) and placed under the control of an SV40e promoter (SEQ ID NO: 60). The EF domains from *C. fumiferana* USP ("CfUSP-EF"; SEQ ID NO: 66) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 51) and placed under the control of an SV40e promoter (SEQ ID NO: 60). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 55) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 61) and placed upstream of the luciferase gene (SEQ ID NO: 62).

1.15 - GAL4DmEcR-DEF/VP16DmUSP-EF: This construct was prepared in the same way as in switch 1.14 above except CfUSP-EF was replaced with the corresponding EF domains from fruit fly *Drosophila melanogaster* USP ("DmUSP-EF", SEQ ID NO: 75).

1.16 - Gal4DmEcR-DEF/VP16LmUSP-EF: This construct was prepared in the same way as in switch 1.14 above except CfUSP-EF was replaced with the EF domains from *Locusta migratoria* USP ("LmUSP-EF"; SEQ ID NO: 21).

1.17 - GAL4DmEcR-DEF/VP16MmRXR $\alpha$ -EF: This construct was prepared in the same way as in switch 1.14 above except CfUSP-EF was replaced with the EF domains of *Mus musculus* MmRXR $\alpha$  ("MmRXR $\alpha$ -EF", SEQ ID NO: 9).

1.18 - GAL4DmEcR-DEF/VP16AmaRXR1-EF: This construct was prepared in the same way as in switch 1.14 above except CfUSP-EF was replaced with the EF domains of ixodid tick *Amblyomma americanum* RXR homolog 1 ("AmaRXR1-EF", SEQ ID NO: 22).

1.19 - GAL4DmEcR-DEF/VP16AmaRXR2-EF: This construct was prepared in the same way as in switch 1.14 above except CfUSP-EF was replaced with the EF domains of ixodid tick *A. americanum* RXR homolog 2 ("AmaRXR2-EF", SEQ ID NO: 23).

1.20 - Gal4DmEcR-DEF/VP16MmRXR $\alpha$ (1-7)-LmUSP(8-12)-EF: This construct was prepared in the same way as in switch 1.14 above except CfUSP-EF was replaced with helices 1 through 7 of MmRXR $\alpha$ -EF and helices 8 through 12 of LmUSP-EF (SEQ ID NO: 45).

1.21 - GAL4TmEcR-DEF/VP16MmRXR $\alpha$ (1-7)-LmUSP(8-12)-EF: This construct was prepared in the same way as in switch 1.20 above except DmEcR-DEF was replaced with the corresponding D, E, and F domains from beetle *Tenebrio molitor* EcR ("TmEcR-DEF", SEQ ID NO: 71), fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 47) and placed under the control of an SV40e promoter (SEQ ID NO: 60). Chimeric EF domains comprising helices 1 through 7 of MmRXR $\alpha$ -EF and helices 8 through 12 of LmUSP-EF (SEQ ID NO: 45) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 51) and placed under the control of an SV40e promoter (SEQ ID NO: 60). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 55) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 61) and placed upstream of the luciferase gene (SEQ ID NO: 62).

1.22 - Gal4AmaEcR-DEF/VP16MmRXR $\alpha$ (1-7)-LmUSP(8-12)-EF: This construct was prepared in the

same way as in switch 1.21 above except TmEcR-DEF was replaced with the corresponding DEF domains of tick *Amblyomma americanum* EcR ("AmaEcR-DEF", SEQ ID NO: 73).

1.23 - GAL4CfEcR-CDEF/VP16HsRXR $\beta$ -EF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-CDEF"; SEQ ID NO: 59) were fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 47) and placed under the control of an SV40e promoter (SEQ ID NO: 60). The EF domains from human *Homo sapiens* RXR $\beta$  ("HsRXR $\beta$ -EF"; SEQ ID NO: 13) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 51) and placed under the control of an SV40e promoter (SEQ ID NO: 60). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 55) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 61) and placed upstream of the luciferase gene (SEQ ID NO: 62).

1.24 - GAL4CfEcR-DEF/VP16HsRXR $\beta$ -EF: This construct was prepared in the same way as in switch 1.23 above except CfEcR-CDEF was replaced with the DEF domains of *C. fumiferana* EcR ("CfEcR-DEF"; SEQ ID NO: 65).

1.25 - GAL4CfEcR-DEF/VP16HsRXR $\beta$ (1-6)-LmUSP(7-12)-EF ("βChimera#6"): This construct was prepared in the same way as in switch 1.24 above except HsRXR $\beta$ -EF was replaced with helices 1 through 6 of HsRXR $\beta$ -EF (nucleotides 1-348 of SEQ ID NO: 13) and helices 7 through 12 of LmUSP-EF (nucleotides 268-630 of SEQ ID NO: 21).

1.26 - GAL4CfEcR-DEF/VP16HsRXR $\beta$ (1-7)-LmUSP(8-12)-EF ("βChimera#8"): This construct was prepared in the same way as in switch 1.24 above except HsRXR $\beta$ -EF was replaced with helices 1 through 7 of HsRXR $\beta$ -EF (nucleotides 1-408 of SEQ ID NO: 13) and helices 8 through 12 of LmUSP-EF (nucleotides 337-630 of SEQ ID NO: 21).

1.27 - GAL4CfEcR-DEF/VP16HsRXR $\beta$ (1-8)-LmUSP(9-12)-EF ("βChimera#9"): This construct was prepared in the same way as in switch 1.24 above except HsRXR $\beta$ -EF was replaced with helices 1 through 8 of HsRXR $\beta$ -EF (nucleotides 1-465 of SEQ ID NO: 13) and helices 9 through 12 of LmUSP-EF (nucleotides 403-630 of SEQ ID NO: 21).

1.28 - GAL4CfEcR-DEF/VP16HsRXR $\beta$ (1-9)-LmUSP(10-12)-EF ("βChimera#10"): This construct was prepared in the same way as in switch 1.24 above except HsRXR $\beta$ -EF was replaced with helices 1 through 9 of HsRXR $\beta$ -EF (nucleotides 1-555 of SEQ ID NO: 13) and helices 10 through 12 of LmUSP-EF (nucleotides 490-630 of SEQ ID NO: 21).

1.29 - GAL4CfEcR-DEF/VP16HsRXR $\beta$ (1-10)-LmUSP(11-12)-EF ("βChimera#11"): This construct was prepared in the same way as in switch 1.24 above except HsRXR $\beta$ -EF was replaced with helices 1 through 10 of HsRXR $\beta$ -EF (nucleotides 1-624 of SEQ ID NO: 13) and helices 11 through 12 of LmUSP-EF (nucleotides 547-630 of SEQ ID NO: 21).

1.30 - GAL4DmEcR-DEF/VP16HsRXR $\beta$ (1-6)-LmUSP(7-12)-EF ("βChimera#6"): This construct was prepared in the same way as in switch 1.25 above except CfEcR-DEF was replaced with DmEcR-DEF

(SEQ ID NO: 67).

1.31 - GAL4DmEcR-DEF/VP16HsRXR $\beta$ (1-7)-LmUSP(8-12)-EF (" $\beta$ Chimera#8"): This construct was prepared in the same way as in switch 1.26 above except CfEcR-DEF was replaced with DmEcR-DEF (SEQ ID NO: 67).

5 1.32 - GAL4DmEcR-DEF/VP16HsRXR $\beta$ (1-8)-LmUSP(9-12)-EF (" $\beta$ Chimera#9"): This construct was prepared in the same way as in switch 1.27 above except CfEcR-DEF was replaced with DmEcR-DEF (SEQ ID NO: 67).

1.33 - GAL4DmEcR-DEF/VP16HsRXR $\beta$ (1-9)-LmUSP(10-12)-EF (" $\beta$ Chimera#10"): This construct was prepared in the same way as in switch 1.28 above CfEcR-DEF was replaced with DmEcR-DEF

10 (SEQ ID NO: 67).

1.34 - GAL4DmEcR-DEF/VP16HsRXR $\beta$ (1-10)-LmUSP(11-12)-EF (" $\beta$ Chimera#11"): This construct was prepared in the same way as in switch 1.29 above except CfEcR-DEF was replaced with DmEcR-DEF (SEQ ID NO: 67).

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## EXAMPLE 2

Applicants have recently made the surprising discovery that invertebrate RXRs and their non-Lepidopteran and non-Dipteran RXR homologs can function similarly to or better than vertebrate RXRs in an ecdysone receptor-based inducible gene expression modulation system in both yeast and  
20 mammalian cells (U.S. provisional application serial No. 60/294,814). Indeed, Applicants have demonstrated that LmUSP is a better partner for CfEcR than mouse RXR in mammalian cells. Yet for most gene expression system applications, particularly those destined for mammalian cells, it is desirable to have a vertebrate RXR as a partner. To identify a minimum region of LmUSP required for this improvement, Applicants have constructed and analyzed vertebrate RXR/invertebrate RXR chimeras  
25 (referred to herein interchangeably as "chimeric RXR's" or "RXR chimeras") in an EcR-based inducible gene expression modulation system. Briefly, gene induction potential (magnitude of induction) and ligand specificity and sensitivity were examined using a non-steroidal ligand in a dose-dependent induction of reporter gene expression in the transfected NIH3T3 cells and A549 cells.

In the first set of RXR chimeras, helices 8 to 12 from MmRXR $\alpha$ -EF were replaced with helices 8  
30 to 12 from LmUSP-EF (switch 1.3 as prepared in Example 1). Three independent clones (RXR chimeras Ch#1, Ch#2, and Ch#3 in Figures 1-3) were picked and compared with the parental MmRXR $\alpha$ -EF and LmUSP-EF switches (switches 1.1 and 1.2, respectively, as prepared in Example 1). The RXR chimera and parent DNAs were transfected into mouse NIH3T3 cells along with Gal4/CfEcR-CDEF and the reporter plasmid pFRLuc. The transfected cells were grown in the presence of 0, 0.2, 1, 5, and 10  $\mu$ M  
35 non-steroidal ligand N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine (GS-E<sup>TM</sup> ligand). The cells were harvested at 48 hours post treatment and the reporter activity was assayed. The numbers on top of bars correspond to the maximum fold activation/induction for that

treatment.

**Transfections:** DNAs corresponding to the various switch constructs outlined in Example 1, specifically switches 1.1 through 1.6 were transfected into mouse NIH3T3 cells (ATCC) and human A549 cells (ATCC) as follows. Cells were harvested when they reached 50% confluency and plated in 5 6-, 12- or 24- well plates at 125,000, 50,000, or 25,000 cells, respectively, in 2.5, 1.0, or 0.5 ml of growth medium containing 10% fetal bovine serum (FBS), respectively. NIH3T3 cells were grown in Dulbecco's modified Eagle medium (DMEM; LifeTechnologies) and A549 cells were grown in F12K nutrient mixture (LifeTechnologies). The next day, the cells were rinsed with growth medium and transfected for four hours. Superfect™ (Qiagen Inc.) was found to be the best transfection reagent for 10 3T3 cells and A549 cells. For 12- well plates, 4 µl of Superfect™ was mixed with 100 µl of growth medium. 1.0 µg of reporter construct and 0.25 µg of each receptor construct of the receptor pair to be analyzed were added to the transfection mix. A second reporter construct was added [pTKRL (Promega), 0.1 µg/transfection mix] that comprises a *Renilla* luciferase gene operably linked and placed under the control of a thymidine kinase (TK) constitutive promoter and was used for normalization. The 15 contents of the transfection mix were mixed in a vortex mixer and let stand at room temperature for 30 min. At the end of incubation, the transfection mix was added to the cells maintained in 400 µl growth medium. The cells were maintained at 37°C and 5% CO<sub>2</sub> for four hours. At the end of incubation, 500 µl of growth medium containing 20% FBS and either dimethylsulfoxide (DMSO; control) or a DMSO solution of 0.2, 1, 5, 10, and 50 µM N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert- 20 butylhydrazine non-steroidal ligand was added and the cells were maintained at 37 °C and 5% CO<sub>2</sub> for 48 hours. The cells were harvested and reporter activity was assayed. The same procedure was followed for 6 and 24 well plates as well except all the reagents were doubled for 6 well plates and reduced to half for 24-well plates.

**Ligand:** The non-steroidal ligand N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-t- 25 butylhydrazine (GS™-E non-steroidal ligand) is a synthetic stable ecdysteroid ligand synthesized at Rohm and Haas Company. Ligands were dissolved in DMSO and the final concentration of DMSO was maintained at 0.1% in both controls and treatments.

**Reporter Assays:** Cells were harvested 48 hours after adding ligands. 125, 250, or 500 µl of passive lysis buffer (part of Dual-luciferase™ reporter assay system from Promega Corporation) were added to 30 each well of 24- or 12- or 6-well plates respectively. The plates were placed on a rotary shaker for 15 minutes. Twenty µl of lysate were assayed. Luciferase activity was measured using Dual-luciferase™ reporter assay system from Promega Corporation following the manufacturer's instructions. β-Galactosidase was measured using Galacto-Star™ assay kit from TROPIX following the manufacturer's instructions. All luciferase and β-galactosidase activities were normalized using *Renilla* luciferase as a 35 standard. Fold activities were calculated by dividing normalized relative light units ("RLU") in ligand treated cells with normalized RLU in DMSO treated cells (untreated control).

**Results:** Surprisingly, all three independent clones of the RXR chimera tested (switch 1.3) were better

than either parent-based switch, MmRXR $\alpha$ -EF (switch 1.1) and LmUSP-EF (switch 1.2), see Figure 1. In particular, the chimeric RXR demonstrated increased ligand sensitivity and increased magnitude of induction. Thus, Applicants have made the surprising discovery that a chimeric RXR ligand binding domain may be used in place of a vertebrate RXR or an invertebrate RXR in an EcR-based inducible gene expression modulation system. This novel EcR/chimeric RXR-based gene expression system provides an improved system characterized by both increased ligand sensitivity and increased magnitude of induction.

The best two RXR chimeras clones of switch 1.3 ("Ch#1" and "Ch#2" of Figure 2) were compared with the parent-based switches 1.1 and 1.2 in a repeated experiment ("Chim-1" and "Chim-2" in Figure 2, respectively). In this experiment, the chimeric RXR-based switch was again more sensitive to non-steroidal ligand than either parent-based switch (see Figure 2). However, in this experiment, the chimeric RXR-based switch was better than the vertebrate RXR (MmRXR $\alpha$ -EF)-based switch for magnitude of induction but was similar to the invertebrate RXR (LmUSP-EF)-based switch.

The same chimeric RXR- and parent RXR-based switches were also examined in a human lung carcinoma cell line A549 (ATCC) and similar results were observed (Figure 3).

Thus, Applicants have demonstrated for the first time that a chimeric RXR ligand binding domain can function effectively in partnership with an ecdysone receptor in an inducible gene expression system in mammalian cells. Surprisingly, the EcR/chimeric RXR-based inducible gene expression system of the present invention is an improvement over both the EcR/vertebrate RXR- and EcR/invertebrate RXR-based gene expression modulation systems since less ligand is required for transactivation and increased levels of transactivation can be achieved.

Based upon Applicant's discovery described herein, one of ordinary skill in the art is able to predict that other chimeric RXR ligand binding domain comprising at least two different species RXR polypeptide fragments from a vertebrate RXR LBD, an invertebrate RXR LBD, or a non-Dipteran and non-Lepidopteran invertebrate RXR homolog will also function in Applicants' EcR/chimeric RXR-based inducible gene expression system. Based upon Applicants' invention, the means to make additional chimeric RXR LBD embodiments within the scope of the present invention is within the art and no undue experimentation is necessary. Indeed, one of skill in the art can routinely clone and sequence a polynucleotide encoding a vertebrate or invertebrate RXR or RXR homolog LBD, and based upon sequence homology analyses similar to that presented in Figure 4, and determine the corresponding polynucleotide and polypeptide fragments of that particular species RXR LBD that are encompassed within the scope of the present invention.

One of ordinary skill in the art is also able to predict that Applicants' novel inducible gene expression system will also work to modulate gene expression in yeast cells. Since the Dipteran RXR homolog/ and Lepidopteran RXR homolog/EcR-based gene expression systems function constitutively in yeast cells (data not shown), similar to how they function in mammalian cells, and non-Dipteran and non-Lepidopteran invertebrate RXRs function inducibly in partnership with an EcR in mammalian cells,



the EcR/chimeric RXR-based inducible gene expression modulation system is predicted to function inducibly in yeast cells, similar to how it functions in mammalian cells. Thus, the EcR/chimeric RXR inducible gene expression system of the present invention is useful in applications where modulation of gene expression levels is desired in both yeast and mammalian cells. Furthermore, Applicants' invention is also contemplated to work in other cells, including but not limited to bacterial cells, fungal cells, and animal cells.

### EXAMPLE 3

There are six amino acids in the C-terminal end of the LBD that are different between MmRXR $\alpha$  and LmUSP (see sequence alignments presented in Figure 4). To verify if these six amino acids contribute to the differences observed between MmRXR $\alpha$  and LmUSP transactivation abilities, Applicants constructed RXR chimeras in which the C-terminal six amino acids, designated herein as the F domain, of one parent RXR were substituted for the F domain of the other parent RXR. Gene switches comprising LmUSP-EF fused to MmRXR $\alpha$ -F (VP16/LmUSP-EF-MmRXR $\alpha$ -F, switch 1.6), MmRXR $\alpha$ -EF fused to LmUSP-F (VP16/MmRXR $\alpha$ -EF-LmUSP-F, switch 1.5), and MmRXR $\alpha$ -EF(1-7)-LmUSP-EF(8-12) fused to MmRXR $\alpha$ -F (Chimera/RXR-F, switch 1.4) were constructed as described in Example 1. These constructs were transfected in NIH3T3 cells and transactivation potential was assayed in the presence of 0, 0.2, 1, and 10  $\mu$ M N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine non-steroidal ligand. The F-domain chimeras (gene switches 1.4-1.6) were compared to the MmRXR $\alpha$ -EF(1-7)-LmUSP-EF(8-12) chimeric RXR LBD of gene switch 1.3. Plasmid pFRLUC (Stratagene) encoding a luciferase polypeptide was used as a reporter gene construct and pTKRL (Promega) encoding a *Renilla* luciferase polypeptide under the control of the constitutive TK promoter was used to normalize the transfections as described above. The cells were harvested, lysed and luciferase reporter activity was measured in the cell lysates. Total fly luciferase relative light units are presented. The number on the top of each bar is the maximum fold induction for that treatment. The analysis was performed in triplicate and mean luciferase counts [total relative light units (RLU)] were determined as described above.

As shown in Figure 5, the six amino acids in the C-terminal end of the LBD (F domain) do not appear to account for the differences observed between vertebrate RXR and invertebrate RXR transactivation abilities, suggesting that helices 8-12 of the EF domain are most likely responsible for these differences between vertebrate and invertebrate RXRs.

### EXAMPLE 4

This Example describes the construction of four EcR-DEF-based gene switches comprising the DEF domains from *Choristoneura fumiferana* (Lepidoptera), *Drosophila melanogaster* (Diptera),

*Tenebrio molitor* (Coleoptera), and *Amblyomma americanum* (Ixodidae) fused to a GAL4 DNA binding domain. In addition, the EF domains of vertebrate RXRs, invertebrate RXRs, or invertebrate USPs from *Choristoneura fumiferana* USP, *Drosophila melanogaster* USP, *Locusta migratoria* USP (Orthoptera), *Mus musculus* RXR $\alpha$  (Vertebrata), a chimera between MmRXR $\alpha$  and LmUSP (Chimera; of switch 1.13), *Amblyomma americanum* RXR homolog 1 (Ixodidae), *Amblyomma americanum* RXR homolog 2 (Ixodidae) were fused to a VP16 activation domain. The receptor combinations were compared for their ability to transactivate the reporter plasmid pFRLuc in mouse NIH3T3 cells in the presence of 0, 0.2, 1, or 10  $\mu$ M PonA steroidal ligand (Sigma Chemical Company) or 0, 0.04, 0.2, 1, or 10  $\mu$ M N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine non-steroidal ligand as described above. The cells were harvested, lysed and luciferase reporter activity was measured in the cell lysates. Total fly luciferase relative light units are presented. The number on the top of each bar is the maximum fold induction for that treatment. The analysis was performed in triplicate and mean luciferase counts [total relative light units (RLU)] were determined as described above.

Figures 6-8 show the results of these analyses. The MmRXR-LmUSP chimera was the best partner for CfEcR (11,000 fold induction, Figure 6), DmEcR (1759 fold induction, Figure 7). For all other EcRs tested, the RXR chimera produced higher background levels in the absence of ligand (see Figure 8). The CfEcR/chimeric RXR-based switch (switch 1.13) was more sensitive to non-steroid than PonA whereas, the DmEcR/chimeric RXR-based switch (switch 1.20) was more sensitive to PonA than non-steroid. Since these two switch formats produce decent levels of induction and show differential sensitivity to steroids and non-steroids, these may be exploited for applications in which two or more gene switches are desired.

Except for CfEcR, all other EcRs tested in partnership the chimeric RXR are more sensitive to steroids than to non-steroids. The TmEcR/chimeric RXR-based switch (switch 1.21; Figure 8) is more sensitive to PonA and less sensitive to non-steroid and works best when partnered with either MmRXR $\alpha$ , AmaRXR1, or AmaRXR2. The AmaEcR/chimeric RXR-based switch (switch 1.22; Figure 8) is also more sensitive to PonA and less sensitive to non-steroid and works best when partnered with either an LmUSP, MmRXR, AmaRXR1 or AmaRXR2-based gene expression cassette. Thus, TmEcR/ and AmaEcR/chimeric RXR-based gene switches appear to form a group of ecdysone receptors that is different from lepidopteran and dipteran EcR/chimeric RXR-based gene switches group (CfEcR/chimeric RXR and DmEcR/chimeric RXR, respectively). As noted above, the differential ligand sensitivities of Applicants' EcR/chimeric RXR-based gene switches are advantageous for use in applications in which two or more gene switches are desired.

## EXAMPLE 5

This Example describes Applicants' further analysis of gene expression cassettes encoding various chimeric RXR polypeptides comprising a mouse RXR $\alpha$  isoform polypeptide fragment or a

human RXR $\beta$  isoform polypeptide fragment and an LmUSP polypeptide fragment in mouse NIH3T3 cells. These RXR chimeras were constructed in an effort to identify the helix or helices of the EF domain that account for the observed transactivational differences between vertebrate and invertebrate RXRs. Briefly, five different gene expression cassettes encoding a chimeric RXR ligand binding domain were constructed as described in Example 1. The five chimeric RXR ligand binding domains encoded by these gene expression cassettes and the respective vertebrate RXR and invertebrate RXR fragments they comprise are depicted in Table 1.

Table 1

10 HsRXR $\beta$ /LmUSP EF Domain Chimeric RXRs

Chimera Name	HsRXR $\beta$ -EF Polypeptide Fragment(s)	LmUSP-EF Polypeptide Fragment(s)
$\beta$ Chimera #6	Helices 1-6	Helices 7-12
$\beta$ Chimera #8	Helices 1-7	Helices 8-12
$\beta$ Chimera #9	Helices 1-8	Helices 9-12
$\beta$ Chimera #10	Helices 1-9	Helices 10-12
$\beta$ Chimera #11	Helices 1-10	Helices 11-12

Three individual clones of each chimeric RXR LBD of Table 1 were transfected into mouse NIH3T3 cells along with either GAL4CfEcR-DEF (switches 1.25-1.29 of Example 1; Figures 9 and 10) or GAL4DmEcR-DEF (switches 1.30-1.34 of Example 1; Figure 11) and the reporter plasmid pFRLuc as described above. The transfected cells were cultured in the presence of either a) 0, 0.2, 1, or 10  $\mu$ M non-steroidal ligand (Figure 9), or b) 0, 0.2, 1, or 10  $\mu$ M steroid ligand PonA or 0, 0.4, 0.2, 1, or 10  $\mu$ M non-steroid ligand (Figures 10 and 11) for 48 hours. The reporter gene activity was measured and total RLU are shown. The number on top of each bar is the maximum fold induction for that treatment and is the mean of three replicates.

20 As shown in Figure 9, the best results were obtained when an HsRXR $\beta$ H1-8 and LmUSP H9-12 chimeric RXR ligand binding domain (of switch 1.27) was used, indicating that helix 9 of LmUSP may be responsible for sensitivity and magnitude of induction.

Using CfEcR as a partner, chimera 9 demonstrated maximum induction (see Figure 10). Chimeras 6 and 8 also produced good induction and lower background, as a result the fold induction was higher for these two chimeras when compared to chimera 9. Chimeras 10 and 11 produced lower levels of reporter activity.

Using DmEcR as a partner, chimera 8 produced the reporter activity (see Figure 11). Chimera 9 also performed well, whereas chimeras 6, 10 and 11 demonstrated lower levels of reporter activity.

The selection of a particular chimeric RXR ligand binding domain can also influence the performance EcR in response to a particular ligand. Specifically, CfEcR in combination with chimera 11

responded well to non-steroid but not to PonA (see Figure 10). Conversely, DmEcR in combination with chimera 11 responded well to PonA but not to non-steroid (see Figure 11).

#### EXAMPLE 6

5

This Example demonstrates the effect of introduction of a second ligand into the host cell comprising an EcR/chimeric RXR-based inducible gene expression modulation system of the invention. In particular, Applicants have determined the effect of 9-cis-retinoic acid on the transactivation potential of the GAL4CfEcR-DEF/VP16HsRXR $\beta$ -(1-8)-LmUSP-(9-12)-EF ( $\beta$ chimera 9; switch 1.27) gene switch  
10 along with pFRLuc in NIH 3T3 cells in the presence of non-steroid (GSE) for 48 hours.

Briefly, GAL4CfEcR-DEF, pFRLuc and VP16HsRXR $\beta$ -(1-8)-LmUSP-(9-12)-EF (chimera #9) were transfected into NIH3T3 cells and the transfected cells were treated with 0, 0.04, 0.2, 1, 5 and 25  $\mu$ M non-steroidal ligand (GSE) and 0, 1, 5 and 25  $\mu$ M 9-cis-retinoic acid (Sigma Chemical Company). The reporter activity was measured at 48 hours after adding ligands.

15 As shown in Figure 12, the presence of retinoic acid increased the sensitivity of CfEcR-DEF to non-steroidal ligand. At a non-steroid ligand concentration of 0.04  $\mu$ M, there is very little induction in the absence of 9-cis-retinoic acid, but when 1  $\mu$ M 9-cis-retinoic acid is added in addition to 0.04  $\mu$ M non-steroid, induction is greatly increased.

WE CLAIM:

1. A gene expression modulation system comprising:
  - a) a first gene expression cassette that is capable of being expressed in a host cell
    - i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
    - ii) an ecdysone receptor ligand binding domain; and
  - b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising:
    - i) a transactivation domain; and
    - ii) a chimeric retinoid X receptor ligand binding domain.
2. The gene expression modulation system according to claim 1, further comprising a third gene expression cassette comprising:
  - i) a response element recognized by the DNA-binding domain of the first hybrid polypeptide;
  - ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and
  - iii) a gene whose expression is to be modulated.
3. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain (LBD) of the first hybrid polypeptide is selected from the group consisting of a spruce budworm *Choristoneura fumiferana* EcR ("CfEcR") LBD, a beetle *Tenebrio molitor* EcR ("TmEcR") LBD, a *Manduca sexta* EcR ("MsEcR") LBD, a *Heliothies virescens* EcR ("HvEcR") LBD, a midge *Chironomus tentans* EcR ("CtEcR") LBD, a silk moth *Bombyx mori* EcR ("BmEcR") LBD, a fruit fly *Drosophila melanogaster* EcR ("DmEcR") LBD, a mosquito *Aedes aegypti* EcR ("AaEcR") LBD, a blowfly *Lucilia capitata* EcR ("LcEcR") LBD, a blowfly *Lucilia cuprina* EcR ("LucEcR") LBD, a Mediterranean fruit fly *Ceratitidis capitata* EcR ("CcEcR") LBD, a locust *Locusta migratoria* EcR ("LmEcR") LBD, an aphid *Myzus persicae* EcR ("MpEcR") LBD, a fiddler crab *Celuca pugilator* EcR ("CpEcR") LBD, a whitefly *Bamecia argentifoli* EcR (BaEcR) LBD, a leafhopper *Nephotetix cincticeps* EcR (NcEcR) LBD, and an ixodid tick *Amblyomma americanum* EcR ("AmaEcR") LBD.
4. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain of the first hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF), SEQ ID NO: 67 (DmEcR-DEF), SEQ ID NO: 71 (TmEcR-DEF) and SEQ ID NO: 73 (AmaEcR-DEF).
5. The gene expression modulation system according to claim 1, wherein the ecdysone

receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 72 (TmEcR-DEF) or SEQ ID NO: 74 (AmaEcR-DEF).

6. The gene expression modulation system according to claim 1, wherein the chimeric  
5 retinoid X receptor ligand binding domain of the second hybrid polypeptide comprises at least two different retinoid X receptor ligand binding domain fragments selected from the group consisting of a vertebrate species retinoid X receptor ligand binding domain fragment, an invertebrate species retinoid X receptor ligand binding domain fragment, and a non-Dipteran/non-Lepidopteran invertebrate species retinoid X receptor homolog ligand binding domain fragment.
- 10 7. The gene expression modulation system according to claim 1, wherein the chimeric retinoid X receptor ligand binding domain of the second hybrid polypeptide comprises a retinoid X receptor ligand binding domain comprising at least one retinoid X receptor ligand binding domain fragment selected from the group consisting of an EF-domain helix 1, an EF-domain helix 2, an EF-domain helix 3, an EF-domain helix 4, an EF-domain helix 5, an EF-domain helix 6, an EF-domain helix  
15 7, an EF-domain helix 8, and EF-domain helix 9, an EF-domain helix 10, an EF-domain helix 11, an EF-domain helix 12, an F-domain, and an EF-domain  $\beta$ -pleated sheet, wherein the retinoid X receptor ligand binding domain fragment is from a different species retinoid X receptor ligand binding domain or a different isoform retinoid X receptor ligand binding domain than the retinoid X receptor ligand binding domain.
- 20 8. The gene expression modulation system according to claim 1, wherein the chimeric retinoid X receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID  
25 NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.
9. The gene expression modulation system according to claim 1, wherein the chimeric  
30 retinoid X receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f)  
35 amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

10. The gene expression modulation system according to claim 1, wherein the first gene expression cassette comprises a polynucleotide sequence that encodes the first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and an ecdysone receptor ligand binding domain.

5 11. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes the second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and a chimeric retinoid X receptor ligand binding domain.

12. The gene expression modulation system according to claim 1, wherein the second gene  
10 expression cassette comprises a polynucleotide that encodes the second hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 51) and a B42 AD (SEQ ID NO: 53), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO:  
15 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO:  
20 13 and nucleotides 613-630 of SEQ ID NO: 21.

13. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes the second hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 52) and a B42 AD (SEQ ID NO: 54), and a chimeric retinoid X receptor ligand  
25 binding domain comprising an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids  
30 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

14. A gene expression modulation system comprising:

a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide sequence that encodes a first hybrid polypeptide comprising:

- 35 i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and  
ii) a chimeric retinoid X receptor ligand binding domain; and

b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising:

- i) a transactivation domain; and
- ii) an ecdysone receptor ligand binding domain.

5        15.     The gene expression modulation system according to claim 14, further comprising a third gene expression cassette comprising:

i) a response element that recognizes the DNA-binding domain of the first hybrid polypeptide;

10        ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and

iii) a gene whose expression is to be modulated.

16.     The gene expression modulation system according to claim 14, wherein the chimeric retinoid X receptor ligand binding domain of the first hybrid polypeptide comprises at least two different retinoid X receptor ligand binding domain fragments selected from the group consisting of a vertebrate  
15 species retinoid X receptor ligand binding domain fragment, an invertebrate species retinoid X receptor ligand binding domain fragment, and a non-Dipteran/non-Lepidopteran invertebrate species retinoid X receptor homolog ligand binding domain fragment.

17.     The gene expression modulation system according to claim 14, wherein the chimeric retinoid X receptor ligand binding domain of the first hybrid polypeptide comprises a retinoid X receptor  
20 ligand binding domain comprising at least one retinoid X receptor ligand binding domain fragment selected from the group consisting of an EF-domain helix 1, an EF-domain helix 2, an EF-domain helix 3, an EF-domain helix 4, an EF-domain helix 5, an EF-domain helix 6, an EF-domain helix 7, an EF-domain helix 8, and EF-domain helix 9, an EF-domain helix 10, an EF-domain helix 11, an EF-domain helix 12, an F-domain, and an EF-domain  $\beta$ -pleated sheet, wherein the retinoid X receptor ligand binding  
25 domain fragment is from a different species retinoid X receptor ligand binding domain or a different isoform retinoid X receptor ligand binding domain than the retinoid X receptor ligand binding domain.

18.     The gene expression modulation system according to claim 14, wherein the chimeric retinoid X receptor ligand binding domain of the first hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b)  
30 nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h)  
35 nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

19.     The gene expression modulation system according to claim 14, wherein the chimeric retinoid X receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid



sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

20. The gene expression modulation system according to claim 14, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF), SEQ ID NO: 67 (DmEcR-DEF), SEQ ID NO: 71 (TmEcR-DEF) and SEQ ID NO: 73 (AmaEcR-DEF).

21. The gene expression modulation system according to claim 14, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 57 (CfEcR-DEF), SEQ ID NO: 58 (DmEcR-DEF), SEQ ID NO: 70 (CfEcR-CDEF), SEQ ID NO: 72 (TmEcR-DEF) or SEQ ID NO: 74 (AmaEcR-DEF).

22. The gene expression modulation system according to claim 14, wherein the first gene expression cassette comprises a polynucleotide that encodes the first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and a chimeric retinoid X receptor ligand binding domain.

23. The gene expression modulation system according to claim 14, wherein the first gene expression cassette comprises a polynucleotide that encodes the first hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 47) and a LexA DBD (SEQ ID NO: 49), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

24. The gene expression modulation system according to claim 14, wherein the first gene expression cassette comprises a polynucleotide that encodes the first hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 48) and a LexA DBD (SEQ ID NO: 50), and a chimeric retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of a) SEQ ID

NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

25. The gene expression modulation system according to claim 14, wherein the second gene expression cassette comprises a polynucleotide that encodes the second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and an ecdysone receptor ligand binding domain.

26. A gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide comprising a DNA-binding domain and a chimeric retinoid X receptor ligand binding domain.

27. The gene expression cassette according to claim 26, wherein the chimeric retinoid X receptor ligand binding domain comprises at least two different retinoid X receptor ligand binding domain fragments selected from the group consisting of a vertebrate species retinoid X receptor ligand binding domain fragment, an invertebrate species retinoid X receptor ligand binding domain fragment, and a non-Dipteran/non-Lepidopteran invertebrate species retinoid X receptor homolog ligand binding domain fragment.

28. The gene expression cassette according to claim 26, wherein the chimeric retinoid X receptor ligand binding domain comprises a retinoid X receptor ligand binding domain comprising at least one retinoid X receptor ligand binding domain fragment selected from the group consisting of an EF-domain helix 1, an EF-domain helix 2, an EF-domain helix 3, an EF-domain helix 4, an EF-domain helix 5, an EF-domain helix 6, an EF-domain helix 7, an EF-domain helix 8, and EF-domain helix 9, an EF-domain helix 10, an EF-domain helix 11, an EF-domain helix 12, an F-domain, and an EF-domain  $\beta$ -pleated sheet, wherein the retinoid X receptor ligand binding domain fragment is from a different species retinoid X receptor ligand binding domain or a different isoform retinoid X receptor ligand binding domain than the retinoid X receptor ligand binding domain.

29. The gene expression cassette according to claim 26, wherein the DNA-binding domain is a GAL4 DNA-binding domain or a LexA DNA-binding domain.

30. The gene expression cassette according to claim 26, wherein the gene expression cassette comprises a polynucleotide encoding a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 47) and a LexA DBD (SEQ ID NO: 49), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides

1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

- 5           31.     The gene expression cassette according to claim 26, wherein the gene expression cassette comprises a polynucleotide encoding a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 48) and a LexA DBD (SEQ ID NO: 50), and a chimeric retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino  
10   acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h)  
15   amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

32.     A gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide comprising a transactivation domain and a chimeric retinoid X receptor ligand binding domain.

33.     The gene expression cassette according to claim 32, wherein the transactivation domain is a VP16 transactivation domain or a B42 acidic activator transactivation domain.

- 20           34.     The gene expression cassette according to claim 32, wherein the gene expression cassette comprises a polynucleotide encoding a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 51) and a B42 AD (SEQ ID NO: 53), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group  
25   consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555 of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides  
30   601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

- 35           35.     The gene expression cassette according to claim 32, wherein the gene expression cassette comprises a polynucleotide encoding a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 52)  
35   and a B42 AD (SEQ ID NO: 54), and a chimeric retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13

and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

36. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces ligand binding activity of the truncated chimeric retinoid X receptor ligand binding domain.

37. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces steroid binding activity of the truncated chimeric retinoid X receptor ligand binding domain.

38. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces non-steroid binding activity of the truncated chimeric retinoid X receptor ligand binding domain.

39. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances ligand binding activity of the truncated chimeric retinoid X receptor ligand binding domain.

40. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances steroid binding activity of the truncated chimeric retinoid X receptor ligand binding domain.

41. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances non-steroid binding activity of the truncated chimeric retinoid X receptor ligand binding domain.

42. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of the truncated chimeric retinoid X receptor ligand binding domain.

43. An isolated polynucleotide encoding a truncated chimeric retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of a heterodimer, wherein the heterodimer comprises the truncated chimeric retinoid X receptor ligand binding domain and a dimerization partner.

44. The isolated polynucleotide according to claim 43, wherein the dimerization partner is an ecdysone receptor polypeptide.

45. An isolated polynucleotide encoding a chimeric retinoid X receptor ligand binding domain, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of a) SEQ ID NO: 45, b) nucleotides 1-348 of SEQ ID NO: 13 and nucleotides 268-630 of SEQ ID NO: 21, c) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, d) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21, e) nucleotides 1-555

of SEQ ID NO: 13 and nucleotides 490-630 of SEQ ID NO: 21, f) nucleotides 1-624 of SEQ ID NO: 13 and nucleotides 547-630 of SEQ ID NO: 21, g) nucleotides 1-645 of SEQ ID NO: 13 and nucleotides 601-630 of SEQ ID NO: 21, and h) nucleotides 1-717 of SEQ ID NO: 13 and nucleotides 613-630 of SEQ ID NO: 21.

5 46. An isolated polypeptide encoded by the isolated polynucleotide according to claim 45.

47. An isolated chimeric retinoid X receptor polypeptide comprising an amino acid sequence selected from the group consisting of a) SEQ ID NO: 46, b) amino acids 1-116 of SEQ ID NO: 13 and amino acids 90-210 of SEQ ID NO: 21, c) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, d) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21, e) amino acids 1-185 of SEQ ID NO: 13 and amino acids 164-210 of SEQ ID NO: 21, f) amino acids 1-208 of SEQ ID NO: 13 and amino acids 183-210 of SEQ ID NO: 21, g) amino acids 1-215 of SEQ ID NO: 13 and amino acids 201-210 of SEQ ID NO: 21, and h) amino acids 1-239 of SEQ ID NO: 13 and amino acids 205-210 of SEQ ID NO: 21.

48. A method of modulating the expression of a gene in a host cell comprising the gene to be modulated comprising the steps of:

a) introducing into the host cell the gene expression modulation system according to claim 1; and

b) introducing into the host cell a ligand;

wherein the gene to be modulated is a component of a gene expression cassette comprising:

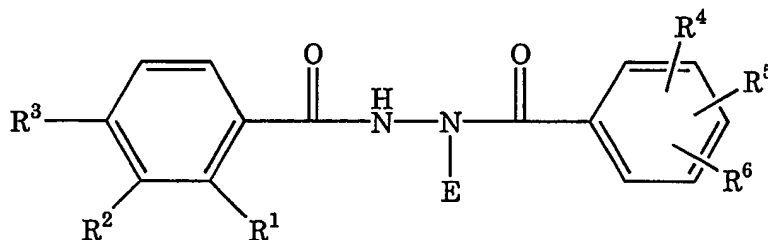
20 i) a response element recognized by the DNA binding domain from the first hybrid polypeptide binds;

ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and

iii) a gene whose expression is to be modulated;

25 whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

49. The method according to claim 48, wherein the ligand is a compound of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

30 R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN,

CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon; R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon; R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

50. The method according to claim 48, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

51. A method of modulating the expression of a gene in a host cell comprising the gene to be modulated comprising the steps of:

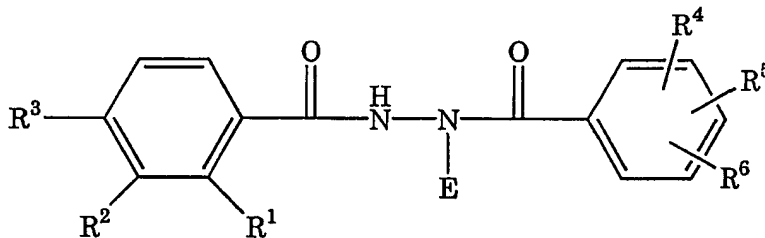
- a) introducing into the host cell the gene expression modulation system of claim 14; and
- b) introducing into the host cell a ligand;

wherein the gene to be modulated is a component of a gene expression cassette comprising:

- i) a response element recognized by the DNA binding domain from the first hybrid polypeptide;
- ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and
- iii) a gene whose expression is to be modulated;

whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

52. The method according to claim 51, wherein the ligand is a compound of the formula:



wherein:

- E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;
- R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;
- R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>,

SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

- 5 R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

- 10 53. The method according to claim 51, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

54. An isolated host cell comprising the gene expression modulation system according to claim 1.

55. The isolated host cell according to claim 54, wherein the host cell is selected from the  
15 group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

56. The isolated host cell according to claim 55, wherein the mammalian cell is a murine cell or a human cell.

57. An isolated host cell comprising the gene expression modulation system according to claim 14.

- 20 58. The isolated host cell according to claim 57, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

59. The isolated host cell according to claim 58, wherein the mammalian cell is a murine cell or a human cell.

60. A non-human organism comprising the host cell of claim 54.

- 25 61. The non-human organism according to claim 60, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.

62. The non-human organism according to claim 61, wherein the mammal is selected from the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, and a chimpanzee.

- 30 63. A non-human organism comprising the host cell of claim 57.

64. The non-human organism according to claim 63, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.

65. The non-human organism according to claim 64, wherein the mammal is selected from the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a  
35 monkey, and a chimpanzee.

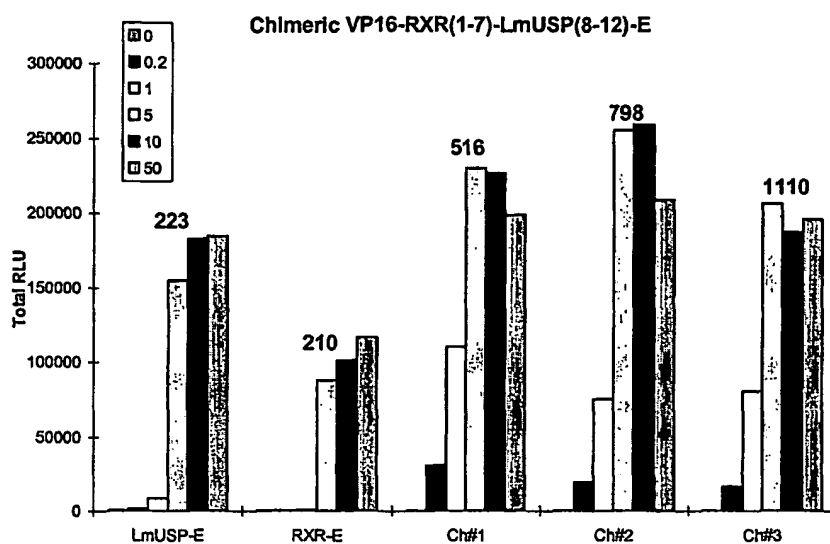


Figure 1

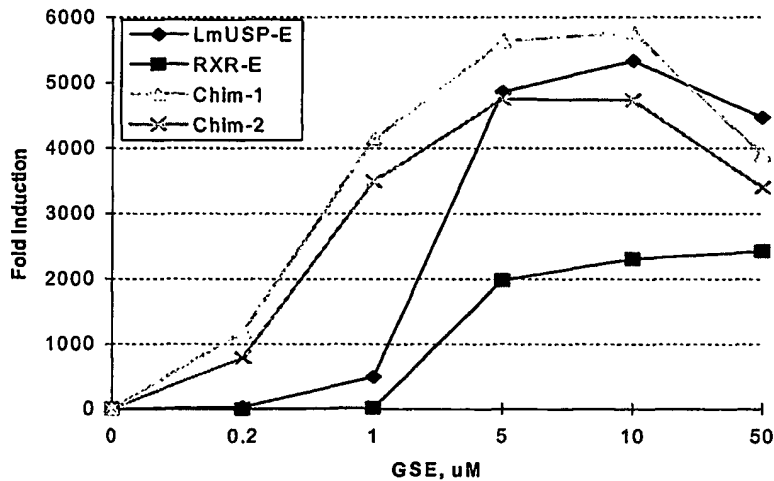


Figure 2



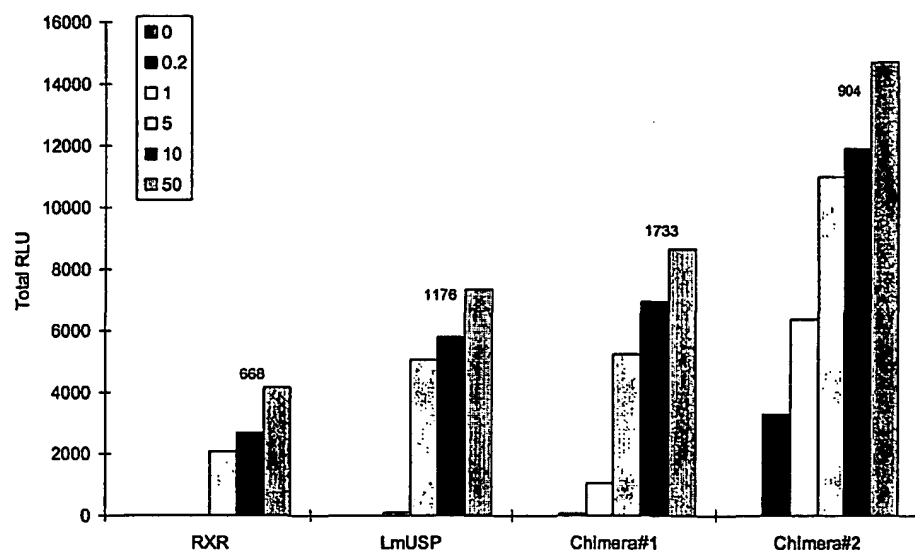


Figure 3

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HsXRaEF ANEDMPVERILEAE LAVEPKTETYVEAN--MGLNPSSPNDPVTNICQAADKQLFTLVWA 58
MmXRaEF ANEDMPVEKILEAE LAVEPKTETYVEAN--MGLNPSSPNDPVTNICQAADKQLFTLVWA 58
HsRXRgEF GHEDMPVERILEAE LAVEPKTESYGDMN----MENSTNDPVTNICHAADKQLFTLVWA 55
MmRXRgEF SHEDMPVERILEAE LAVEPKTESYGDMN----VENSTNDPVTNICHAADKQLFTLVWA 55
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HsRXRbEF KRIPHFSSPLDDQVILLRAGWNELLIASFSHRSIDVRDGLLATGLHVHRNSAHSAGVG 120
MmRXRbEF KRIPHFSSPLDDQVILLRAGWNELLIASFSHRSIDVRDGLLATGLHVHRNSAHSAGVG 120
HsXRaEF KRIPHFSELPLDDQVILLRAGWNELLIASFSHRSIAVKDGLLATGLHVHRNSAHSAGVG 118
MmXRaEF KRIPHFSELPLDDQVILLRAGWNELLIASFSHRSIAVKDGLLATGLHVHRNSAHSAGVG 118
HsRXRgEF KRIPHFSDLTLEDQVILLRAGWNELLIASFSHRSVSVQDGLLATGLHVHRSSAHSAGVG 115
MmRXRgEF KRIPHFSDLTLEDQVILLRAGWNELLIASFSHRSVSVQDGLLATGLHVHRSSAHSAGVG 115
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      B8A1      B9
HsRXRbEF AIFDRVLTTELVS KM RDM RMD TELGCLRAIILFNPDAGKLSNPSEVEVLREKVYASLETY 180
MmRXRbEF AIFDRVLTTELVS KM RDM RMD TELGCLRAIIMFNPDAGKLSNPGEVEILREKVYASLETY 180
HsXRaEF AIFDRVLTTELVS KM RDM QMD TELGCLRAIVLFNPD SKGLSNPAEVEALREKVYASLEAY 178
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      B10      B11
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HsXRaEF CKHKYPEQGRFAKLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQMT 237
MmXRaEF CKHKYPEQGRFAKLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQAT 237
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      H10      H11      H12      F

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Figure 4A

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CpRXREF -SDMPIASIREAELSVDPIDEQPLDQGVRVLPAPPDSEKCSFTLPFPVSEVSCANPL 59
AmaRXR1EF PPEMLERILEAELRVES-QTGTLSSES-----AQQ- 29
AmaRXR2EF SPDMPLEIRILEAEMRVEQPAPSVLAQT-----AASG 31
      H1

LmRXREF E-----LVEWAKHIPHFTSLPLEDQVLLLRAGWNELLIAAFSHRSVDVK 70
AmRXREF ENAVSHICNATNKQLFQLVAWAKHIPHFTSLPLEDQVLLLRAGWNELLIASFSHRSIDVK 86
TmRXREF HDGVNNICQATNKQLFQLVQWAKLIPHFTSLPMSDQVLLLRAGWNELLIAAFSHRSIQAQ 89
CpRXREF QDVVSNICQAADRLVQLVEWAKHIPHFTDLPIEDQVLLKAGWNELLIASFSHRSMGVE 119
AmaRXR1EF QDPVSSICQAADRLVQLVQWAKLIPHFELPLEDRMVLLKAGWNELLIAAFSHRSVDVR 89
AmaRXR2EF RDPVNSMCQAAP-PLHELQWARRIPHFEELPIEDRTALLKAGWNELLIAAFSHRSVAVR 90
      H3      H4      H5

      B6      B8 A1      B9
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      B10      B11
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CpRXREF GLNCVNDVEILREKVYAALSEYTRTTHPDEPCRFKLLRLPALRSIGLKCLEHLFFFRL 239
AmaRXR1EF GLRTCPSGGPEGESV-SALEEHCRQYYPDQPCRFKLLRLPALRSIGLKCLEHLFFFRL 208
AmaRXR2EF GLRNATRVLEALREKVYAALSEHCRRHHPDQPCRFKLLRLPALRSIGLKCLEHLFFFRL 210
      H9      H10      H11

LmRXREF IGDVPIDTFLMEMLESPPSDS----- 210
AmRXREF IGDVPIDDFLVEMLESRSDP----- 226
TmRXREF IGDVPIDTFLMEMLESPPADA----- 229
CpRXREF IGDTPIDSYLMKMLVDNPNTSVTPPTS 266
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AmaRXR2EF IGDTPIDSFLLNMLEAPADP----- 230
      H12 F

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Figure 4B

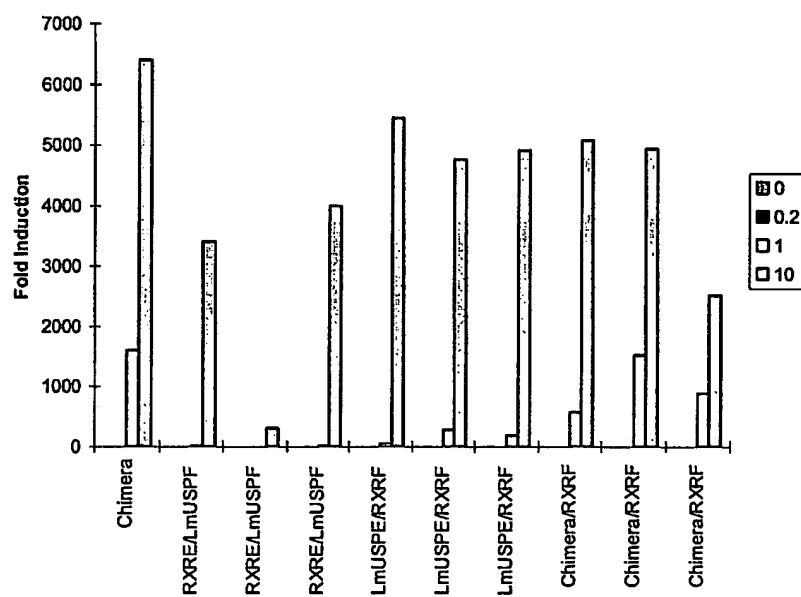


Figure 5

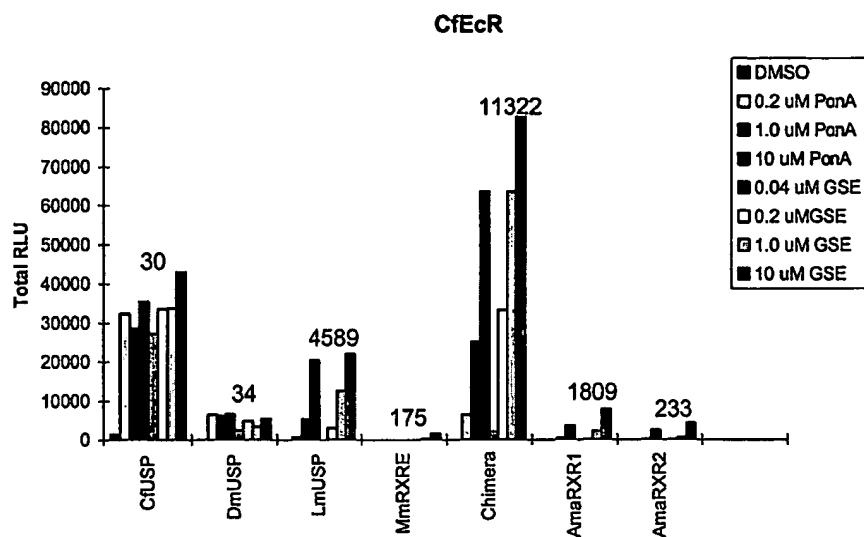


Figure 6

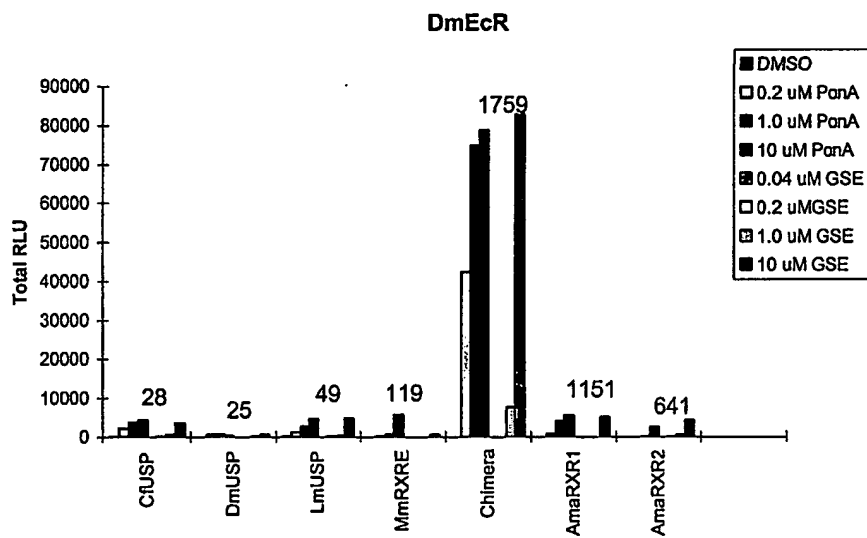


Figure 7

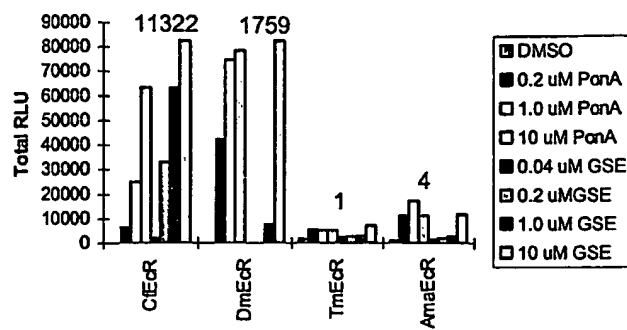


Figure 8

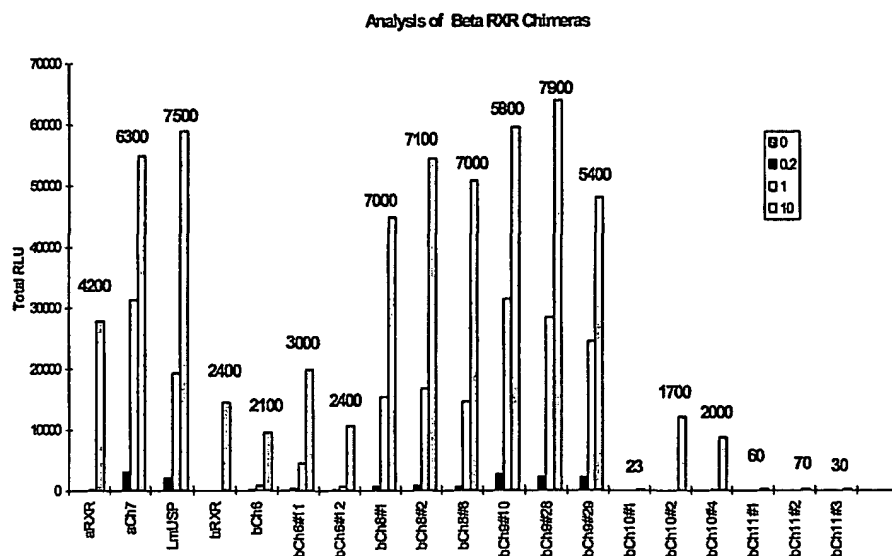


Figure 9

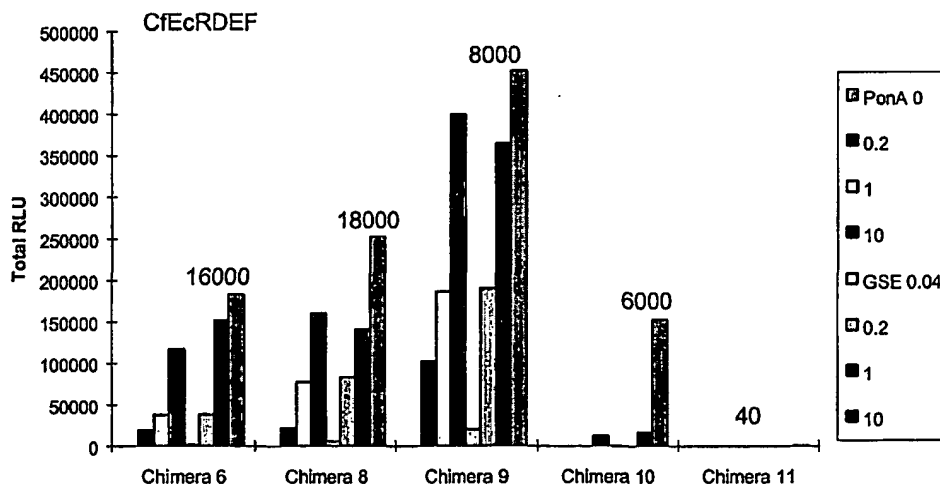


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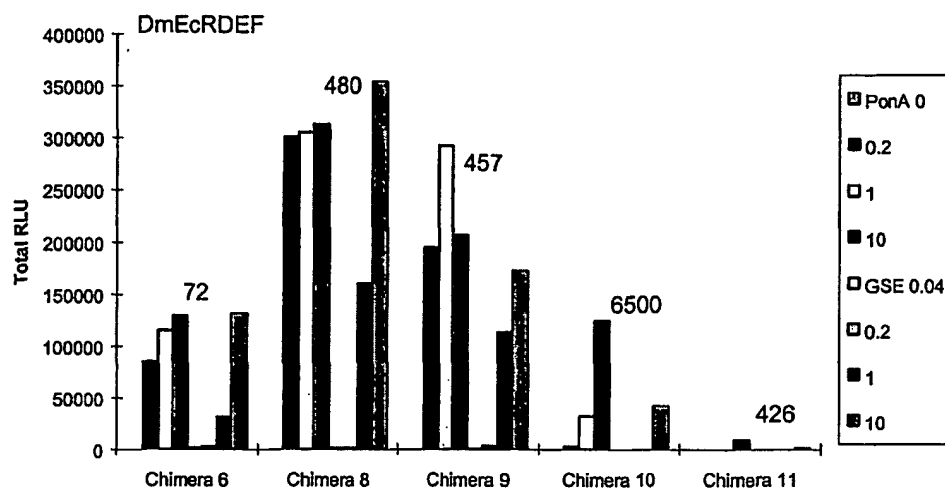


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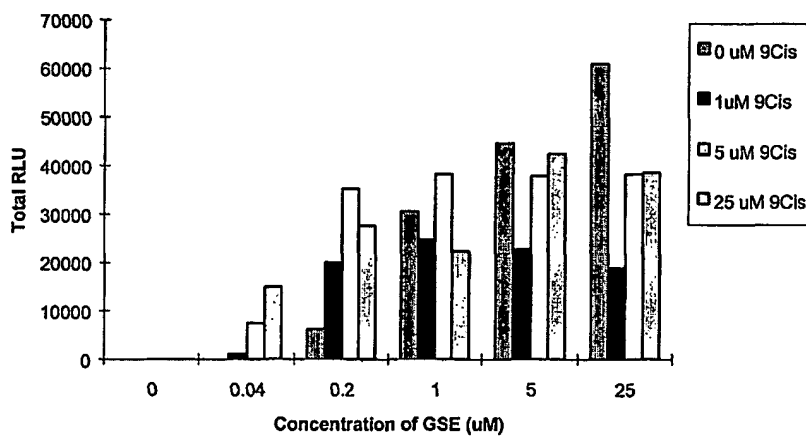


Figure 12



## SEQUENCE LISTING

<110> Rohm and Haas Company  
 Palli, Subba R.  
 Kapitskaya, Marianna Z.

<120> Chimeric retinoid X receptors and their use in a novel ecdysone receptor-based inducible gene expression system

<130> A01238

<150> US 60/294,819

<151> 2001-05-31

<160> 75

<170> PatentIn version 3.1

<210> 1

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<212> DNA

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atctcgcagc ctgatcaaat tacgctgctt aaggcttgct caagtgaggt aatgatgctc	240
cgagtcgcgc gacgatacga tgcggcctca gacagtgttc tggtcgcgaa caaccaagcg	300
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gactctgcct ccacttcggc ggcggcagcc gcggcccagc atcagcctca gcctcagccc 840
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caacctcagc taccacctca gctgcaaggt caactgcaac cccagctcca accacagctt 960
cagacgcaac tccagccaca gattcaacca cagccacagc tcttcccggt ctccgctccc 1020
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&lt;210&gt; 3

&lt;211&gt; 960

&lt;212&gt; DNA

<213> *Choristoneura fumiferana*

&lt;400&gt; 3

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atgcagtgtg aacctccacc tctgaagca gcaaggattc acgaagtggc cccaagggtt 180
ctctccgaca agctgttggg gacaaaccgg cagaaaaaca tccccagtt gacagccaac 240
cagcagttcc ttatcgccag gctcatctgg taccaggacg ggtacgagca gccttctgat 300
gaagatttga agaggattac gcagacgtgg cagcaagcgg acgatgaaaa cgaagagtct 360

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gacactccct tccgccagat cacagagatg actatcctca cgggccaaact tatcgtggag 420  
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<210> 4

<211> 969

<212> DNA

<213> *Drosophila melanogaster*

<400> 4

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 gagccgcccc agcatgccac tattccgcta ctacctgatg aaatattggc caagtgtcaa 240  
 gcgcgcaata taccttcctt aacgtacaat cagttggccg ttatatacaa gttaatttgg 300  
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 <211> 244  
 <212> PRT  
 <213> Choristoneura fumiferana  
 <400> 5

Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile  
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Thr Gln Thr Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr  
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Pro Phe Arg Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile  
 35 40 45

Val Glu Phe Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro  
 50 55 60

Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu  
 65 70 75 80

Arg Val Ala Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val Leu Phe Ala  
 85 90 95

Asn Asn Gln Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala  
 100 105 110

Tyr Val Ile Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met  
 115 120 125

Ala Leu Asp Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe  
 130 135 140

Ser Asp Arg Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln  
 145 150 155 160

Arg Tyr Tyr Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser  
 165 170 175

Gly Ser Ala Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu  
 180 185 190

Ser Glu Leu Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser  
 195 200 205

Leu Lys Leu Lys Asn Arg Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp

210                      215                      220  
 Asp Val Ala Asp Met Ser His Thr Gln Pro Pro Pro Ile Leu Glu Ser  
 225                      230                      235                      240  
  
 Pro Thr Asn Leu  
  
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 <211> 445  
 <212> PRT  
 <213> Drosophila melanogaster  
  
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                     20                      25                      30  
  
 Ile Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly Leu  
                     35                      40                      45  
  
 Pro Ala Phe Thr Lys Ile Pro Gln Glu Asp Gln Ile Thr Leu Leu Lys  
                     50                      55                      60  
  
 Ala Cys Ser Ser Glu Val Met Met Leu Arg Met Ala Arg Arg Tyr Asp  
 65                      70                      75                      80  
  
 His Ser Ser Asp Ser Ile Phe Phe Ala Asn Asn Arg Ser Tyr Thr Arg  
                     85                      90                      95  
  
 Asp Ser Tyr Lys Met Ala Gly Met Ala Asp Asn Ile Glu Asp Leu Leu  
                     100                      105                      110  
  
 His Phe Cys Arg Gln Met Phe Ser Met Lys Val Asp Asn Val Glu Tyr  
                     115                      120                      125  
  
 Ala Leu Leu Thr Ala Ile Val Ile Phe Ser Asp Arg Pro Gly Leu Glu  
                     130                      135                      140  
  
 Lys Ala Gln Leu Val Glu Ala Ile Gln Ser Tyr Tyr Ile Asp Thr Leu  
 145                      150                      155                      160  
  
 Arg Ile Tyr Ile Leu Asn Arg His Cys Gly Asp Ser Met Ser Leu Val  
                     165                      170                      175

Phe Tyr Ala Lys Leu Leu Ser Ile Leu Thr Glu Leu Arg Thr Leu Gly  
 180 185 190

Asn Gln Asn Ala Glu Met Cys Phe Ser Leu Lys Leu Lys Asn Arg Lys  
 195 200 205

Leu Pro Lys Phe Leu Glu Glu Ile Trp Asp Val His Ala Ile Pro Pro  
 210 215 220

Ser Val Gln Ser His Leu Gln Ile Thr Gln Glu Glu Asn Glu Arg Leu  
 225 230 235 240

Glu Arg Ala Glu Arg Met Arg Ala Ser Val Gly Gly Ala Ile Thr Ala  
 245 250 255

Gly Ile Asp Cys Asp Ser Ala Ser Thr Ser Ala Ala Ala Ala Ala Ala  
 260 265 270

Gln His Gln Pro Gln Pro Gln Pro Gln Pro Gln Pro Ser Ser Leu Thr  
 275 280 285

Gln Asn Asp Ser Gln His Gln Thr Gln Pro Gln Leu Gln Pro Gln Leu  
 290 295 300

Pro Pro Gln Leu Gln Gly Gln Leu Gln Pro Gln Leu Gln Pro Gln Leu  
 305 310 315 320

Gln Thr Gln Leu Gln Pro Gln Ile Gln Pro Gln Pro Gln Leu Leu Pro  
 325 330 335

Val Ser Ala Pro Val Pro Ala Ser Val Thr Ala Pro Gly Ser Leu Ser  
 340 345 350

Ala Val Ser Thr Ser Ser Glu Tyr Met Gly Gly Ser Ala Ala Ile Gly  
 355 360 365

Pro Ile Thr Pro Ala Thr Thr Ser Ser Ile Thr Ala Ala Val Thr Ala  
 370 375 380

Ser Ser Thr Thr Ser Ala Val Pro Met Gly Asn Gly Val Gly Val Gly  
 385 390 395 400

Val Gly Val Gly Gly Asn Val Ser Met Tyr Ala Asn Ala Gln Thr Ala  
 405 410 415

Met Ala Leu Met Gly Val Ala Leu His Ser His Gln Glu Gln Leu Ile  
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Gly Gly Val Ala Val Lys Ser Glu His Ser Thr Thr Ala  
                   435                  440                  445

<210> 7  
 <211> 320  
 <212> PRT  
 <213> Choristoneura fumiferana

<400> 7

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Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser Thr Thr Thr  
                   20                  25                  30

Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro Pro Pro Pro  
                   35                  40                  45

Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu Ser Asp Lys  
                   50                  55                  60

Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu Thr Ala Asn  
 65                  70                  75                  80

Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp Gly Tyr Glu  
                   85                  90                  95

Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr Trp Gln Gln  
                   100                  105                  110

Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg Gln Ile Thr  
                   115                  120                  125

Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly  
                   130                  135                  140

Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile Thr Leu Leu  
 145                  150                  155                  160

Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala Arg Arg Tyr  
                   165                  170                  175

Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln Ala Tyr Thr  
                   180                  185                  190

Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile Glu Asp Leu  
 195 200 205

Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp Asn Ile His  
 210 215 220

Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg Pro Gly Leu  
 225 230 235 240

Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr Leu Asn Thr  
 245 250 255

Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala Arg Ser Ser  
 260 265 270

Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu Arg Thr Leu  
 275 280 285

Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu Lys Asn Arg  
 290 295 300

Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala Asp Met Ser  
 305 310 315 320

<210> 8  
 <211> 323  
 <212> PRT  
 <213> Drosophila melanogaster

<400> 8

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Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Met Thr Thr Ser Pro Ser  
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Ser Gln His Gly Gly Asn Gly Ser Leu Ala Ser Gly Gly Gly Gln Asp  
 35 40 45

Phe Val Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu Pro Pro Gln  
 50 55 60

His Ala Thr Ile Pro Leu Leu Pro Asp Glu Ile Leu Ala Lys Cys Gln  
 65 70 75 80



Ala Arg Asn Ile Pro Ser Leu Thr Tyr Asn Gln Leu Ala Val Ile Tyr  
                             85                            90                            95

Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Glu Glu Asp  
                             100                            105                            110

Leu Arg Arg Ile Met Ser Gln Pro Asp Glu Asn Glu Ser Gln Thr Asp  
                             115                            120                            125

Val Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr Val Gln Leu  
                             130                            135                            140

Ile Val Glu Phe Ala Lys Gly Leu Pro Ala Phe Thr Lys Ile Pro Gln  
                             145                            150                            155                            160

Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met  
                             165                            170                            175

Leu Arg Met Ala Arg Arg Tyr Asp His Ser Ser Asp Ser Ile Phe Phe  
                             180                            185                            190

Ala Asn Asn Arg Ser Tyr Thr Arg Asp Ser Tyr Lys Met Ala Gly Met  
                             195                            200                            205

Ala Asp Asn Ile Glu Asp Leu Leu His Phe Cys Arg Gln Met Phe Ser  
                             210                            215                            220

Met Lys Val Asp Asn Val Glu Tyr Ala Leu Leu Thr Ala Ile Val Ile  
                             225                            230                            235                            240

Phe Ser Asp Arg Pro Gly Leu Glu Lys Ala Gln Leu Val Glu Ala Ile  
                             245                            250                            255

Gln Ser Tyr Tyr Ile Asp Thr Leu Arg Ile Tyr Ile Leu Asn Arg His  
                             260                            265                            270

Cys Gly Asp Ser Met Ser Leu Val Phe Tyr Ala Lys Leu Leu Ser Ile  
                             275                            280                            285

Leu Thr Glu Leu Arg Thr Leu Gly Asn Gln Asn Ala Glu Met Cys Phe  
                             290                            295                            300

Ser Leu Lys Leu Lys Asn Arg Lys Leu Pro Lys Phe Leu Glu Glu Ile  
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Trp Asp Val

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 <211> 714  
 <212> DNA  
 <213> Mus musculus

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<210> 10  
 <211> 720  
 <212> DNA  
 <213> Mus musculus

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<210> 11  
 <211> 705  
 <212> DNA  
 <213> Mus musculus

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<210> 12  
 <211> 850  
 <212> DNA  
 <213> Homo sapiens

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<210> 13  
 <211> 720  
 <212> DNA  
 <213> Homo sapiens

<400> 13  
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<210> 14  
 <211> 705  
 <212> DNA  
 <213> Homo sapiens

<400> 14  
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 ctgattgcct ctttctccca ccgctcagtt tccgtgcagg atggcatcct tctggccacg 300  
 ggtttacatg tccaccggag cagtgccac agtgctgggg tcggctccat ctttgacaga 360  
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ccggaacagc caggcaggtt tgccaagctg ctgctgcgcc tcccagctct gcgttcatt    600
ggcttgaaat gcctggagca cctcttcttc ttcaagctca tcggggacac cccattgac    660
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<210> 15
<211> 237
<212> PRT
<213> Mus musculus

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<400> 15

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Ala Asn Glu Asp Met Pro Val Glu Lys Ile Leu Glu Ala Glu Leu Ala
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Val Glu Pro Lys Thr Glu Thr Tyr Val Glu Ala Asn Met Gly Leu Asn
              20              25              30

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Pro Ser Ser Pro Asn Asp Pro Val Thr Asn Ile Cys Gln Ala Ala Asp
              35              40              45

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Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro His Phe
50              55              60

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Ser Glu Leu Pro Leu Asp Asp Gln Val Ile Leu Leu Arg Ala Gly Trp
65              70              75              80

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Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Ile Ala Val Lys
              85              90              95

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Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Asn Ser Ala
100              105              110

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His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg Val Leu Thr Glu Leu
115              120              125

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Val Ser Lys Met Arg Asp Met Gln Met Asp Lys Thr Glu Leu Gly Cys
130              135              140

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Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ser Lys Gly Leu Ser Asn
145              150              155              160

```

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Pro Ala Glu Val Glu Ala Leu Arg Glu Lys Val Tyr Ala Ser Leu Glu
165              170              175

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Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln Pro Gly Arg Phe Ala Lys  
 180 185 190

Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu  
 195 200 205

Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile Asp Thr  
 210 215 220

Phe Leu Met Glu Met Leu Glu Ala Pro His Gln Ala Thr  
 225 230 235

<210> 16  
 <211> 239  
 <212> PRT  
 <213> Mus musculus

<400> 16

Ala Pro Glu Glu Met Pro Val Asp Arg Ile Leu Glu Ala Glu Leu Ala  
 1 5 10 15

Val Glu Gln Lys Ser Asp Gln Gly Val Glu Gly Pro Gly Ala Thr Gly  
 20 25 30

Gly Gly Gly Ser Ser Pro Asn Asp Pro Val Thr Asn Ile Cys Gln Ala  
 35 40 45

Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro  
 50 55 60

His Phe Ser Ser Leu Pro Leu Asp Asp Gln Val Ile Leu Leu Arg Ala  
 65 70 75 80

Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Ile Asp  
 85 90 95

Val Arg Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Asn  
 100 105 110

Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg Val Leu Thr  
 115 120 125

Glu Leu Val Ser Lys Met Arg Asp Met Arg Met Asp Lys Thr Glu Leu  
 130 135 140

Gly Cys Leu Arg Ala Ile Ile Met Phe Asn Pro Asp Ala Lys Gly Leu  
145 150 155 160

Ser Asn Pro Gly Glu Val Glu Ile Leu Arg Glu Lys Val Tyr Ala Ser  
165 170 175

Leu Glu Thr Tyr Cys Lys Gln Lys Tyr Pro Glu Gln Gln Gly Arg Phe  
180 185 190

Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys  
195 200 205

Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile  
210 215 220

Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His Gln Leu Ala  
225 230 235

<210> 17  
<211> 234  
<212> PRT  
<213> Mus musculus

<400> 17

Ser His Glu Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Leu Ala  
1 5 10 15

Val Glu Pro Lys Thr Glu Ser Tyr Gly Asp Met Asn Val Glu Asn Ser  
20 25 30

Thr Asn Asp Pro Val Thr Asn Ile Cys His Ala Ala Asp Lys Gln Leu  
35 40 45

Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro His Phe Ser Asp Leu  
50 55 60

Thr Leu Glu Asp Gln Val Ile Leu Leu Arg Ala Gly Trp Asn Glu Leu  
65 70 75 80

Leu Ile Ala Ser Phe Ser His Arg Ser Val Ser Val Gln Asp Gly Ile  
85 90 95

Leu Leu Ala Thr Gly Leu His Val His Arg Ser Ser Ala His Ser Arg  
100 105 110

Gly Val Gly Ser Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ser Lys  
115 120 125

Met Lys Asp Met Gln Met Asp Lys Ser Glu Leu Gly Cys Leu Arg Ala  
 130 135 140

Ile Val Leu Phe Asn Pro Asp Ala Lys Gly Leu Ser Asn Pro Ser Glu  
 145 150 155 160

Val Glu Thr Leu Arg Glu Lys Val Tyr Ala Thr Leu Glu Ala Tyr Thr  
 165 170 175

Lys Gln Lys Tyr Pro Glu Gln Pro Gly Arg Phe Ala Lys Leu Leu Leu  
 180 185 190

Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu  
 195 200 205

Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile Asp Ser Phe Leu Met  
 210 215 220

Glu Met Leu Glu Thr Pro Leu Gln Ile Thr  
 225 230

<210> 18  
 <211> 237  
 <212> PRT  
 <213> Homo sapiens

<400> 18

Ala Asn Glu Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Leu Ala  
 1 5 10 15

Val Glu Pro Lys Thr Glu Thr Tyr Val Glu Ala Asn Met Gly Leu Asn  
 20 25 30

Pro Ser Ser Pro Asn Asp Pro Val Thr Asn Ile Cys Gln Ala Ala Asp  
 35 40 45

Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro His Phe  
 50 55 60

Ser Glu Leu Pro Leu Asp Asp Gln Val Ile Leu Leu Arg Ala Gly Trp  
 65 70 75 80

Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Ile Ala Val Lys  
 85 90 95



Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Asn Ser Ala  
 100 105 110

His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg Val Leu Thr Glu Leu  
 115 120 125

Val Ser Lys Met Arg Asp Met Gln Met Asp Lys Thr Glu Leu Gly Cys  
 130 135 140

Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ser Lys Gly Leu Ser Asn  
 145 150 155 160

Pro Ala Glu Val Glu Ala Leu Arg Glu Lys Val Tyr Ala Ser Leu Glu  
 165 170 175

Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln Pro Gly Arg Phe Ala Lys  
 180 185 190

Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu  
 195 200 205

Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile Asp Thr  
 210 215 220

Phe Leu Met Glu Met Leu Glu Ala Pro His Gln Met Thr  
 225 230 235

<210> 19  
 <211> 239  
 <212> PRT  
 <213> Homo sapiens

<400> 19

Ala Pro Glu Glu Met Pro Val Asp Arg Ile Leu Glu Ala Glu Leu Ala  
 1 5 10 15

Val Glu Gln Lys Ser Asp Gln Gly Val Glu Gly Pro Gly Gly Thr Gly  
 20 25 30

Gly Ser Gly Ser Ser Pro Asn Asp Pro Val Thr Asn Ile Cys Gln Ala  
 35 40 45

Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro  
 50 55 60

His Phe Ser Ser Leu Pro Leu Asp Asp Gln Val Ile Leu Leu Arg Ala  
 65 70 75 80

Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Ile Asp  
85 90 95

Val Arg Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Asn  
100 105 110

Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg Val Leu Thr  
115 120 125

Glu Leu Val Ser Lys Met Arg Asp Met Arg Met Asp Lys Thr Glu Leu  
130 135 140

Gly Cys Leu Arg Ala Ile Ile Leu Phe Asn Pro Asp Ala Lys Gly Leu  
145 150 155 160

Ser Asn Pro Ser Glu Val Glu Val Leu Arg Glu Lys Val Tyr Ala Ser  
165 170 175

Leu Glu Thr Tyr Cys Lys Gln Lys Tyr Pro Glu Gln Gln Gly Arg Phe  
180 185 190

Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys  
195 200 205

Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile  
210 215 220

Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His Gln Leu Ala  
225 230 235

<210> 20

<211> 234

<212> PRT

<213> Homo sapiens

<400> 20

Gly His Glu Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Leu Ala  
1 5 10 15

Val Glu Pro Lys Thr Glu Ser Tyr Gly Asp Met Asn Met Glu Asn Ser  
20 25 30

Thr Asn Asp Pro Val Thr Asn Ile Cys His Ala Ala Asp Lys Gln Leu  
35 40 45

Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro His Phe Ser Asp Leu  
50 55 60

Thr Leu Glu Asp Gln Val Ile Leu Leu Arg Ala Gly Trp Asn Glu Leu  
65 70 75 80

Leu Ile Ala Ser Phe Ser His Arg Ser Val Ser Val Gln Asp Gly Ile  
85 90 95

Leu Leu Ala Thr Gly Leu His Val His Arg Ser Ser Ala His Ser Ala  
100 105 110

Gly Val Gly Ser Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ser Lys  
115 120 125

Met Lys Asp Met Gln Met Asp Lys Ser Glu Leu Gly Cys Leu Arg Ala  
130 135 140

Ile Val Leu Phe Asn Pro Asp Ala Lys Gly Leu Ser Asn Pro Ser Glu  
145 150 155 160

Val Glu Thr Leu Arg Glu Lys Val Tyr Ala Thr Leu Glu Ala Tyr Thr  
165 170 175

Lys Gln Lys Tyr Pro Glu Gln Pro Gly Arg Phe Ala Lys Leu Leu Leu  
180 185 190

Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu  
195 200 205

Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile Asp Thr Phe Leu Met  
210 215 220

Glu Met Leu Glu Thr Pro Leu Gln Ile Thr  
225 230

<210> 21  
<211> 635  
<212> DNA  
<213> Locusta migratoria

<400> . 21  
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cagaaaacca agtgggaatat gagctggtgg agtgggctaa acacatcccg cacttcacat 120  
ccctacctct ggaggaccag gttctcctcc tcagagcagg ttggaatgaa ctgctaattg 180  
cagcatttttc acatcgatct gtagatgtta aagatggcat agtacttgcc actggtctca 240

cagtgcacgc aaattctgcc catcaagctg gagtcggcac aatatttgac agagttttga 300  
 cagaactggg agcaaagatg agagaaatga aaatggataa aactgaactt ggctgcttgc 360  
 gatctgttat tcttttcaat ccagaggtga ggggtttgaa atccgcccag gaagttgaac 420  
 ttctacgtga aaaagtatat gccgctttgg aagaatatac tagaacaaca catcccgatg 480  
 aaccaggaag atttgcaaaa cttttgcttc gtctgccttc tttacgttcc ataggcctta 540  
 agtggttgga gcatttgctt ttctttcgcc ttattggaga tgtccaatt gatacgttcc 600  
 tgatggagat gcttgaatca ccttctgatt cataa 635

<210> 22  
 <211> 687  
 <212> DNA  
 <213> Amblyomma americanum

<400> 22  
 cctcctgaga tgcctctgga gcgcatactg gaggcagagc tgcgggttga gtcacagacg 60  
 gggaccctct cggaagcgc acagcagcag gatccagtga gcagcatctg ccaagctgca 120  
 gaccgacagc tgcaccagct agttcaatgg gccaagcaca ttccacattt tgaagagctt 180  
 ccccttgagg accgcatggg gttgctcaag gctggctgga acgagctgct cattgctgct 240  
 ttctcccacc gttctgttga cgtgcgtgat ggcatgtgc tcgctacagg tcttggtgtg 300  
 cagcggcata gtgctcatgg ggctggcgtt ggggccatat ttgatagggt tctcactgaa 360  
 ctggtagcaa agatgcgtga gatgaagatg gaccgactg agcttggtat cctgcttgct 420  
 gtggtacttt ttaatcctga ggccaagggg ctgcggacct gccaagtgg aggccttgag 480  
 ggagaaagtg tatctgcctt ggaagagcac tgccggcagc agtaccaga ccagcctggg 540  
 cgctttgcca agctgctgct gcggttgcca gctctgcga gtattggcct caagtgcctc 600  
 gaacatctct ttttcttcaa gctcatcggg gacacgcca tcgacaactt tcttctttcc 660  
 atgctggagg cccctctga cccctaa 687

<210> 23  
 <211> 693  
 <212> DNA  
 <213> Amblyomma americanum

<400> 23  
 tctccggaca tgccactcga acgcattctc gaagccgaga tgcgctcga gcagccggca 60  
 ccgtccgttt tggcgagac ggccgcatcg ggccgcgacc ccgtcaacag catgtgccag 120  
 gctgccccgc cacttcacga gctcgtacag tgggcccggc gaattccga cttcgaagag 180  
 cttcccatcg aggatcgac cgcgtgctc aaagccggct ggaacgaact gcttattgcc 240

gccttttcgc accgtttctgt ggcgggtgcgc gacggcatcg ttctggccac cgggctggtg 300  
gtgcagcggc acagcgcaca cggcgcaggc gttggcgaca tcttcgaccg cgtactagcc 360  
gagctggtgg ccaagatgcg cgacatgaag atggacaaaa cggagctcgg ctgcctgcgc 420  
gccgtggtgc tcttcaatcc agacgccaaag ggtctccgaa acgccaccag agtagaggcg 480  
ctccgcgaga aggtgtatgc ggcgctggag gagcactgcc gtcggcacca cccggaccaa 540  
ccgggtcgct tcggcaagct gctgctgcgg ctgcctgcct tgcgcagcat cgggctcaaa 600  
tgcctcgagc atctgttctt cttcaagctc atcggagaca ctcccataga cagcttcctg 660  
ctcaacatgc tggaggcacc ggcagacccc tag 693

<210> 24  
<211> 801  
<212> DNA  
<213> *Celuca pugilator*

<400> 24  
tcagacatgc caattgccag catacgggag gcagagctca gcgtagatcc catagatgag 60  
cagccgctgg accaaggggt gaggcttcag gttccactcg cacctcctga tagtgaaaag 120  
tgtagcttta ctttaccttt tcatcccgtc agtgaagtat cctgtgctaa ccctctgcag 180  
gatgtggtga gcaacatatg ccaggcagct gacagacatc tggtagcagct ggtggagtgg 240  
gccaaacaca tcccacactt cacagacctt cccatagagg accaagtggg attactcaaa 300  
gccgggtgga acgagttgct tattgcctca ttctcacacc gtagcatggg cgtggaggat 360  
ggcatcgtgc tggccacagg gctcgtgatc cacagaagta gtgctcacca ggctggagtg 420  
ggtgccatat ttgatcgtgt cctctctgag ctggtggcca agatgaagga gatgaagatt 480  
gacaagacag agctgggctg ccttcgctcc atcgtcctgt tcaaccaga tgccaaagga 540  
ctaaactgcg tcaatgatgt ggagatcttg cgtgagaagg tgtatgctgc cctggaggag 600  
tacacacgaa ccacttacc tgatgaacct ggacgctttg ccaagttgct tctgcgactt 660  
cctgcactca ggtctatagg cctgaagtgt cttgagtacc tcttcctgtt taagctgatt 720  
ggagacactc ccctggacag ctacttgatg aagatgctcg tagacaaccc aaatacaagc 780  
gtcactcccc ccaccagcta g 801

<210> 25  
<211> 690  
<212> DNA  
<213> *Tenebrio molitor*

<400> 25  
gccgagatgc ccctcgacag gataatcgag gcggagaaac ggatagaatg cacacccgct 60  
ggtggctctg gtggtgtcgg agagcaacac gacggggtga acaacatctg tcaagccact 120

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aacaagcagc tgttccaact ggtgcaatgg gctaagctca tacctcactt tacctcgttg      180
ccgatgtcgg accaggtgct tttattgagg gcaggatgga atgaattgct catcgccgca      240
ttctcgcaca gatctataca ggcgaggat gccatcgttc tagccacggg gttgacagtt      300
aacaaaacgt cggcgcacgc cgtgggctg ggcaacatct acgaccgct cctctccgag      360
ctgggtgaaca agatgaaaga gatgaagatg gacaagacgg agctgggctg cttgagagcc      420
atcatcctct acaacccac gtgtcgcggc atcaagtccg tgcaggaagt ggagatgctg      480
cgtgagaaaa ttacggcgt gctggaagag tacaccagga ccaccaccc gaacgagccc      540
ggcaggttcg ccaaactgct tctgcgcctc ccggccctca ggtccatcgg gttgaaatgt      600
tccgaacacc tctttttctt caagctgacg ggtgatgttc caatagacac gttcctgatg      660
gagatgctgg agtctccggc ggacgcttag                                     690

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<210> 26
<211> 681
<212> DNA
<213> Apis mellifera

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<400> 26
cattcggaca tgccgatcga gcgtatcctg gaggccgaga agagagtcga atgtaagatg      60
gagcaacagg gaaattacga gaatgcagtg tcgcacattt gcaacgccac gaacaaacag      120
ctgttccagc tggtagcatg ggcgaaacac atcccgcat t tacctcgtt gccactggag      180
gatcaggtag ttctgctcag ggccgggttg aacgagttgc tgatagcctc cttttccac      240
cgttccatcg acgtgaagga cggtatcgtg ctggcgacgg ggatcacctg gcatcggaac      300
tcggcgagcagg agggcggcgt gggcacgata ttgacccgtg tcctctcggg gcttgtctcg      360
aaaatgcgtg aaatgaagat ggacaggaca gagcttggtg gtctcagatc tataatactc      420
ttcaatcccg aggttcgagg actgaaatcc atccaggaag tgaccctgct ccgtgagaag      480
atctacggcg ccctggaggg ttattgcccgc gtagcttggt cgcagcagcg tgggaagattc      540
gcgaaattac ttctacgcct gcccgccatc cgtcgcagcg gattaaagtg cctcgagtag      600
ctgttcttct tcaaaatgat cggtgacgta ccgatcgacg attttctcgt ggagatgtta      660
gaatcgcgat cagatcctta g                                     681

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<210> 27
<211> 210
<212> PRT
<213> Locusta migratoria

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<400> 27

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His Thr Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Lys Arg Val



Pro Pro Glu Met Pro Leu Glu Arg Ile Leu Glu Ala Glu Leu Arg Val  
 1 5 10 15  
 Glu Ser Gln Thr Gly Thr Leu Ser Glu Ser Ala Gln Gln Gln Asp Pro  
 20 25 30  
 Val Ser Ser Ile Cys Gln Ala Ala Asp Arg Gln Leu His Gln Leu Val  
 35 40 45  
 Gln Trp Ala Lys His Ile Pro His Phe Glu Glu Leu Pro Leu Glu Asp  
 50 55 60  
 Arg Met Val Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala  
 65 70 75 80  
 Phe Ser His Arg Ser Val Asp Val Arg Asp Gly Ile Val Leu Ala Thr  
 85 90 95  
 Gly Leu Val Val Gln Arg His Ser Ala His Gly Ala Gly Val Gly Ala  
 100 105 110  
 Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met  
 115 120 125  
 Lys Met Asp Arg Thr Glu Leu Gly Cys Leu Leu Ala Val Val Leu Phe  
 130 135 140  
 Asn Pro Glu Ala Lys Gly Leu Arg Thr Cys Pro Ser Gly Gly Pro Glu  
 145 150 155 160  
 Gly Glu Ser Val Ser Ala Leu Glu Glu His Cys Arg Gln Gln Tyr Pro  
 165 170 175  
 Asp Gln Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu  
 180 185 190  
 Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu  
 195 200 205  
 Ile Gly Asp Thr Pro Ile Asp Asn Phe Leu Leu Ser Met Leu Glu Ala  
 210 215 220  
 Pro Ser Asp Pro  
 225



<210> 29  
 <211> 230  
 <212> PRT  
 <213> Amblyomma americanum

<400> 29

Ser Pro Asp Met Pro Leu Glu Arg Ile Leu Glu Ala Glu Met Arg Val  
 1 5 10 15

Glu Gln Pro Ala Pro Ser Val Leu Ala Gln Thr Ala Ala Ser Gly Arg  
 20 25 30

Asp Pro Val Asn Ser Met Cys Gln Ala Ala Pro Pro Leu His Glu Leu  
 35 40 45

Val Gln Trp Ala Arg Arg Ile Pro His Phe Glu Glu Leu Pro Ile Glu  
 50 55 60

Asp Arg Thr Ala Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala  
 65 70 75 80

Ala Phe Ser His Arg Ser Val Ala Val Arg Asp Gly Ile Val Leu Ala  
 85 90 95

Thr Gly Leu Val Val Gln Arg His Ser Ala His Gly Ala Gly Val Gly  
 100 105 110

Asp Ile Phe Asp Arg Val Leu Ala Glu Leu Val Ala Lys Met Arg Asp  
 115 120 125

Met Lys Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Val Val Leu  
 130 135 140

Phe Asn Pro Asp Ala Lys Gly Leu Arg Asn Ala Thr Arg Val Glu Ala  
 145 150 155 160

Leu Arg Glu Lys Val Tyr Ala Ala Leu Glu Glu His Cys Arg Arg His  
 165 170 175

His Pro Asp Gln Pro Gly Arg Phe Gly Lys Leu Leu Leu Arg Leu Pro  
 180 185 190

Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe  
 195 200 205

Lys Leu Ile Gly Asp Thr Pro Ile Asp Ser Phe Leu Leu Asn Met Leu  
 210 215 220

Glu Ala Pro Ala Asp Pro  
225 230

<210> 30  
<211> 266  
<212> PRT  
<213> Celuca pugilator

<400> 30

Ser Asp Met Pro Ile Ala Ser Ile Arg Glu Ala Glu Leu Ser Val Asp  
1 5 10 15

Pro Ile Asp Glu Gln Pro Leu Asp Gln Gly Val Arg Leu Gln Val Pro  
20 25 30

Leu Ala Pro Pro Asp Ser Glu Lys Cys Ser Phe Thr Leu Pro Phe His  
35 40 45

Pro Val Ser Glu Val Ser Cys Ala Asn Pro Leu Gln Asp Val Val Ser  
50 55 60

Asn Ile Cys Gln Ala Ala Asp Arg His Leu Val Gln Leu Val Glu Trp  
65 70 75 80

Ala Lys His Ile Pro His Phe Thr Asp Leu Pro Ile Glu Asp Gln Val  
85 90 95

Val Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser  
100 105 110

His Arg Ser Met Gly Val Glu Asp Gly Ile Val Leu Ala Thr Gly Leu  
115 120 125

Val Ile His Arg Ser Ser Ala His Gln Ala Gly Val Gly Ala Ile Phe  
130 135 140

Asp Arg Val Leu Ser Glu Leu Val Ala Lys Met Lys Glu Met Lys Ile  
145 150 155 160

Asp Lys Thr Glu Leu Gly Cys Leu Arg Ser Ile Val Leu Phe Asn Pro  
165 170 175

Asp Ala Lys Gly Leu Asn Cys Val Asn Asp Val Glu Ile Leu Arg Glu  
180 185 190

Lys Val Tyr Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr Tyr Pro Asp  
 195 200 205

Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg  
 210 215 220

Ser Ile Gly Leu Lys Cys Leu Glu Tyr Leu Phe Leu Phe Lys Leu Ile  
 225 230 235 240

Gly Asp Thr Pro Leu Asp Ser Tyr Leu Met Lys Met Leu Val Asp Asn  
 245 250 255

Pro Asn Thr Ser Val Thr Pro Pro Thr Ser  
 260 265

<210> 31  
 <211> 229  
 <212> PRT  
 <213> Tenebrio molitor

<400> 31

Ala Glu Met Pro Leu Asp Arg Ile Ile Glu Ala Glu Lys Arg Ile Glu  
 1 5 10 15

Cys Thr Pro Ala Gly Gly Ser Gly Gly Val Gly Glu Gln His Asp Gly  
 20 25 30

Val Asn Asn Ile Cys Gln Ala Thr Asn Lys Gln Leu Phe Gln Leu Val  
 35 40 45

Gln Trp Ala Lys Leu Ile Pro His Phe Thr Ser Leu Pro Met Ser Asp  
 50 55 60

Gln Val Leu Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala  
 65 70 75 80

Phe Ser His Arg Ser Ile Gln Ala Gln Asp Ala Ile Val Leu Ala Thr  
 85 90 95

Gly Leu Thr Val Asn Lys Thr Ser Ala His Ala Val Gly Val Gly Asn  
 100 105 110

Ile Tyr Asp Arg Val Leu Ser Glu Leu Val Asn Lys Met Lys Glu Met  
 115 120 125

Lys Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu Tyr  
 130 135 140

Asn Pro Thr Cys Arg Gly Ile Lys Ser Val Gln Glu Val Glu Met Leu  
 145 150 155 160

Arg Glu Lys Ile Tyr Gly Val Leu Glu Glu Tyr Thr Arg Thr Thr His  
 165 170 175

Pro Asn Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala  
 180 185 190

Leu Arg Ser Ile Gly Leu Lys Cys Ser Glu His Leu Phe Phe Phe Lys  
 195 200 205

Leu Ile Gly Asp Val Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu  
 210 215 220

Ser Pro Ala Asp Ala  
 225

<210> 32  
 <211> 226  
 <212> PRT  
 <213> Apis mellifera

<400> 32

His Ser Asp Met Pro Ile Glu Arg Ile Leu Glu Ala Glu Lys Arg Val  
 1 5 10 15

Glu Cys Lys Met Glu Gln Gln Gly Asn Tyr Glu Asn Ala Val Ser His  
 20 25 30

Ile Cys Asn Ala Thr Asn Lys Gln Leu Phe Gln Leu Val Ala Trp Ala  
 35 40 45

Lys His Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu  
 50 55 60

Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His  
 65 70 75 80

Arg Ser Ile Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Ile Thr  
 85 90 95

Val His Arg Asn Ser Ala Gln Gln Ala Gly Val Gly Thr Ile Phe Asp  
 100 105 110

Arg Val Leu Ser Glu Leu Val Ser Lys Met Arg Glu Met Lys Met Asp  
 115 120 125

Arg Thr Glu Leu Gly Cys Leu Arg Ser Ile Ile Leu Phe Asn Pro Glu  
 130 135 140

Val Arg Gly Leu Lys Ser Ile Gln Glu Val Thr Leu Leu Arg Glu Lys  
 145 150 155 160

Ile Tyr Gly Ala Leu Glu Gly Tyr Cys Arg Val Ala Trp Pro Asp Asp  
 165 170 175

Ala Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Ile Arg Ser  
 180 185 190

Ile Gly Leu Lys Cys Leu Glu Tyr Leu Phe Phe Phe Lys Met Ile Gly  
 195 200 205

Asp Val Pro Ile Asp Asp Phe Leu Val Glu Met Leu Glu Ser Arg Ser  
 210 215 220

Asp Pro  
 225

<210> 33  
 <211> 516  
 <212> DNA  
 <213> Locusta migratoria

<400> 33  
 atccctacct ctggaggacc aggttctcct cctcagagca ggttggaatg aactgctaata 60  
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 cacagtgcac cgaaattctg cccatcaagc tggagtcggc acaatatttg acagagtttt 180  
 gacagaactg gtagcaaaga tgagagaaat gaaaatggat aaaactgaac ttggctgctt 240  
 gcgatctggt attctttttca atccagaggt gagggggttg aaatccgccc aggaagtga 300  
 acttctacgt gaaaaagtat atgccgcttt ggaagaatat actagaacaa cacatccga 360  
 tgaaccagga agatttgcaa aacttttgct tcgtctgcct tctttaogtt ccataggcct 420  
 taagtgtttg gagcatttgt tttctttcgc cttattggag atgttccaat tgatacgttc 480  
 ctgatggaga tgcttgaatc accttctgat tcataa 516

<210> 34  
 <211> 528  
 <212> DNA  
 <213> Amblyomma americanum

<400> 34  
 attccacatt ttgaagagct tccccttgag gaccgcatgg tgttgctcaa ggctggctgg 60  
 aacgagctgc tcattgctgc tttctccac cgttctgttg acgtgctga tggcattgtg 120  
 ctcgctacag gtcttggtgc gcagcggcat agtgctcatg gggctggcgt tggggccata 180  
 tttgataggg ttctcactga actggtagca aagatgctg agatgaagat ggaccgact 240  
 gagcttggat gcctgcttgc tgtggtactt tttaatcctg aggccaagg gctgctggacc 300  
 tgcccaagtg gaggccctga gggagaaagt gtatctgcct tggaagagca ctgccggcag 360  
 cagtaccag accagcctgg gcgctttgcc aagctgctgc tgcggttgc agctctgcgc 420  
 agtattggcc tcaagtgcct cgaacatctc tttttcttca agctcatcgg ggacacgccc 480  
 atcgacaact ttcttcttct catgctggag gccccctctg acccctaa 528

<210> 35  
 <211> 531  
 <212> DNA  
 <213> Amblyomma americanum

<400> 35  
 attccgcact tcgaagagct tcccatcgag gatcgacccg cgctgctcaa agccggctgg 60  
 aacgaactgc ttattgcgc cttttcgcac cgttctgttg cggcgcgga cggcatcgtt 120  
 ctggccaccg ggctgggtgg gcagcggcac agcgcacacg gcgcaggcgt tggcgacatc 180  
 ttgcaccgcg tactagccga gctggtggcc aagatgcgcg acatgaagat ggacaaaacg 240  
 gagctcggct gcctgcgcgc cgtggtgctc ttcaatccag acgccaaggg tctccgaaac 300  
 gccaccagag tagaggcgct ccgcgagaag gtgtatgcgg cgctggagga gcactgccgt 360  
 cggcaccacc cggaccaacc gggtcgcttc ggcaagctgc tgctgcggct gcctgccttg 420  
 cgcagcatcg ggctcaaagt cctcgagcat ctgttcttct tcaagctcat cggagacact 480  
 cccatagaca gcttctgct caacatgctg gaggcaccgg cagacccta g 531

<210> 36  
 <211> 552  
 <212> DNA  
 <213> Celuca pugilator

<400> 36  
 atccacact tcacagacct tcccatagag gaccaagtgg tattactcaa agccgggtgg 60  
 aacgagttgc ttattgcctc attctcacac cgtagcatgg gcgtggagga tggcatcgtg 120  
 ctggccacag ggctcgtgat ccacagaagt agtgctcacc aggctggagt gggtgccata 180  
 tttgatcgtg tcctctctga gctggtggcc aagatgaagg agatgaagat tgacaagaca 240  
 gagctgggct gccttcgctc catcgctctg ttcaaccag atgccaaagg actaaactgc 300

gtcaatgatg tggagatcctt gcgtgagaag gtgtatgctg ccctggagga gtacacacga 360  
 accacttacc ctgatgaacc tggacgcttt gccaaagttgc ttctgcgact tctgcactc 420  
 aggtctatag gcctgaagtg tcttgagtac ctcttcctgt ttaagctgat tggagacact 480  
 ccctggaca gctacttgat gaagatgctc gtagacaacc caaatacaag cgtcactccc 540  
 cccaccagct ag 552

<210> 37  
 <211> 531  
 <212> DNA  
 <213> *Tenebrio molitor*

<400> 37  
 atacctcact ttacctcggt gccgatgtcg gaccagggtgc ttttattgag ggcaggatgg 60  
 aatgaattgc tcatcgccgc attctcgcac agatctatac aggcgcagga tgccatcggt 120  
 ctagccacgg ggttgacagt taacaaaacg tcggcgacgc ccgtgggcgt gggcaacatc 180  
 tacgaccgcg tcctctccga gctggtgaac aagatgaaag agatgaagat ggacaagacg 240  
 gagctgggct gcttgagagc catcatcctc tacaaccca cgtgtcgcgg catcaagtcc 300  
 gtgcaggaag tggagatgct gcgtgagaaa atttacggcg tgctggaaga gtacaccagg 360  
 accaccaccc cgaacgagcc cggcagggtc gccaaactgc ttctgcgct cccggccctc 420  
 aggtccatcg ggttgaaatg ttccgaacac ctcttttct tcaagctgat cggatgatgt 480  
 ccaatagaca cgttcctgat ggagatgctg gagtctccgg cggacgctta g 531

<210> 38  
 <211> 531  
 <212> DNA  
 <213> *Apis mellifera*

<400> 38  
 atccccgatt ttacctcggt gccactggag gatcagggtac ttctgctcag ggccggttgg 60  
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 ctggcgacgg ggatcaccgt gcatcggaac tcggcgacgc aggcggcggt gggcacgata 180  
 ttgaccgtg tcctctccga gcttgtctcg aaaatgcgtg aaatgaagat ggacaggaca 240  
 gagcttggct gtctcagatc tataatactc ttcaatcccg aggttcgagg actgaaatcc 300  
 atccaggaag tgaccctgct ccgtgagaag atctacggcg ccctggaggg ttattgccgc 360  
 gtagcttggc ccgacgacgc tggaagattc gcgaaattac ttctacgct gcccgccatc 420  
 cgctcgatcg gattaaagt cctcgagtac ctgttctct tcaaatgat cggtgacgta 480  
 ccgatcgacg attttctcgt ggagatgtta gaatcgcgat cagatcctta g 531

<210> 39  
 <211> 176  
 <212> PRT  
 <213> *Locusta migratoria*

<400> 39

Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu Leu Leu  
 1 5 10 15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser  
 20 25 30

Val Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Leu Thr Val His  
 35 40 45

Arg Asn Ser Ala His Gln Ala Gly Val Gly Thr Ile Phe Asp Arg Val  
 50 55 60

Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp Lys Thr  
 65 70 75 80

Glu Leu Gly Cys Leu Arg Ser Val Ile Leu Phe Asn Pro Glu Val Arg  
 85 90 95

Gly Leu Lys Ser Ala Gln Glu Val Glu Leu Leu Arg Glu Lys Val Tyr  
 100 105 110

Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asp Glu Pro Gly  
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ser Leu Arg Ser Ile Gly  
 130 135 140

Leu Lys Cys Leu Glu His Leu Phe Phe Phe Arg Leu Ile Gly Asp Val  
 145 150 155 160

Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ser Asp Ser  
 165 170 175

<210> 40  
 <211> 175  
 <212> PRT  
 <213> *Amblyomma americanum*

<400> 40

Ile Pro His Phe Glu Glu Leu Pro Leu Glu Asp Arg Met Val Leu Leu  
 1 5 10 15



Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser  
20 25 30

Val Asp Val Arg Asp Gly Ile Val Leu Ala Thr Gly Leu Val Val Gln  
35 40 45

Arg His Ser Ala His Gly Ala Gly Val Gly Ala Ile Phe Asp Arg Val  
50 55 60

Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp Arg Thr  
65 70 75 80

Glu Leu Gly Cys Leu Leu Ala Val Val Leu Phe Asn Pro Glu Ala Lys  
85 90 95

Gly Leu Arg Thr Cys Pro Ser Gly Gly Pro Glu Gly Glu Ser Val Ser  
100 105 110

Ala Leu Glu Glu His Cys Arg Gln Gln Tyr Pro Asp Gln Pro Gly Arg  
115 120 125

Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu  
130 135 140

Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro  
145 150 155 160

Ile Asp Asn Phe Leu Leu Ser Met Leu Glu Ala Pro Ser Asp Pro  
165 170 175

<210> 41

<211> 176

<212> PRT

<213> Amblyomma americanum

<400> 41

Ile Pro His Phe Glu Glu Leu Pro Ile Glu Asp Arg Thr Ala Leu Leu  
1 5 10 15

Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser  
20 25 30

Val Ala Val Arg Asp Gly Ile Val Leu Ala Thr Gly Leu Val Val Gln  
35 40 45

Arg His Ser Ala His Gly Ala Gly Val Gly Asp Ile Phe Asp Arg Val  
50 55 60

Leu Ala Glu Leu Val Ala Lys Met Arg Asp Met Lys Met Asp Lys Thr  
65 70 75 80

Glu Leu Gly Cys Leu Arg Ala Val Val Leu Phe Asn Pro Asp Ala Lys  
85 90 95

Gly Leu Arg Asn Ala Thr Arg Val Glu Ala Leu Arg Glu Lys Val Tyr  
100 105 110

Ala Ala Leu Glu Glu His Cys Arg Arg His His Pro Asp Gln Pro Gly  
115 120 125

Arg Phe Gly Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly  
130 135 140

Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr  
145 150 155 160

Pro Ile Asp Ser Phe Leu Leu Asn Met Leu Glu Ala Pro Ala Asp Pro  
165 170 175

<210> 42  
<211> 183  
<212> PRT  
<213> Celuca pugilator

<400> 42

Ile Pro His Phe Thr Asp Leu Pro Ile Glu Asp Gln Val Val Leu Leu  
1 5 10 15

Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser  
20 25 30

Met Gly Val Glu Asp Gly Ile Val Leu Ala Thr Gly Leu Val Ile His  
35 40 45

Arg Ser Ser Ala His Gln Ala Gly Val Gly Ala Ile Phe Asp Arg Val  
50 55 60

Leu Ser Glu Leu Val Ala Lys Met Lys Glu Met Lys Ile Asp Lys Thr  
65 70 75 80

Glu Leu Gly Cys Leu Arg Ser Ile Val Leu Phe Asn Pro Asp Ala Lys  
85 90 95

Gly Leu Asn Cys Val Asn Asp Val Glu Ile Leu Arg Glu Lys Val Tyr  
 100 105 110

Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr Tyr Pro Asp Glu Pro Gly  
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly  
 130 135 140

Leu Lys Cys Leu Glu Tyr Leu Phe Leu Phe Lys Leu Ile Gly Asp Thr  
 145 150 155 160

Pro Leu Asp Ser Tyr Leu Met Lys Met Leu Val Asp Asn Pro Asn Thr  
 165 170 175

Ser Val Thr Pro Pro Thr Ser  
 180

<210> 43  
 <211> 176  
 <212> PRT  
 <213> Tenebrio molitor

<400> 43

Ile Pro His Phe Thr Ser Leu Pro Met Ser Asp Gln Val Leu Leu Leu  
 1 5 10 15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser  
 20 25 30

Ile Gln Ala Gln Asp Ala Ile Val Leu Ala Thr Gly Leu Thr Val Asn  
 35 40 45

Lys Thr Ser Ala His Ala Val Gly Val Gly Asn Ile Tyr Asp Arg Val  
 50 55 60

Leu Ser Glu Leu Val Asn Lys Met Lys Glu Met Lys Met Asp Lys Thr  
 65 70 75 80

Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu Tyr Asn Pro Thr Cys Arg  
 85 90 95

Gly Ile Lys Ser Val Gln Glu Val Glu Met Leu Arg Glu Lys Ile Tyr  
 100 105 110

Gly Val Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asn Glu Pro Gly  
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly  
 130 135 140

Leu Lys Cys Ser Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Val  
 145 150 155 160

Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ala Asp Ala  
 165 170 175

<210> 44

<211> 176

<212> PRT

<213> Apis mellifera

<400> 44

Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu Leu Leu  
 1 5 10 15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser  
 20 25 30

Ile Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Ile Thr Val His  
 35 40 45

Arg Asn Ser Ala Gln Gln Ala Gly Val Gly Thr Ile Phe Asp Arg Val  
 50 55 60

Leu Ser Glu Leu Val Ser Lys Met Arg Glu Met Lys Met Asp Arg Thr  
 65 70 75 80

Glu Leu Gly Cys Leu Arg Ser Ile Ile Leu Phe Asn Pro Glu Val Arg  
 85 90 95

Gly Leu Lys Ser Ile Gln Glu Val Thr Leu Leu Arg Glu Lys Ile Tyr  
 100 105 110

Gly Ala Leu Glu Gly Tyr Cys Arg Val Ala Trp Pro Asp Asp Ala Gly  
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Ile Arg Ser Ile Gly  
 130 135 140

Leu Lys Cys Leu Glu Tyr Leu Phe Phe Phe Lys Met Ile Gly Asp Val  
 145 150 155 160

Pro Ile Asp Asp Phe Leu Val Glu Met Leu Glu Ser Arg Ser Asp Pro  
 165 170 175

<210> 45  
 <211> 711  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Chimeric RXR ligand binding domain

<400> 45  
 gccaacgagg acatgcctgt agagaagatt ctggaagccg agcttgctgt cgagcccaag 60  
 actgagacat acgtggaggc aaacatgggg ctgaacccca gctcaccaaa tgaccctgtt 120  
 accaacatct gtcaagcagc agacaagcag ctcttcactc ttgtggagtg ggccaagagg 180  
 atccccacact tttctgagct gccctagac gaccaggtea tcctgctacg ggcaggctgg 240  
 aacgagctgc tgaatgcctc cttctcccac cgctccatag ctgtgaaaga tgggattctc 300  
 ctggccaccg gcctgcacgt acaccggaac agcgctcaca gtgctggggg gggcgccatc 360  
 tttgacaggg tgctaacaga gctggtgtct aagatgcgtg acatgcagat ggacaagact 420  
 gaacttggtc gcttgcatc tgttattctt ttcaatccag aggtgagggg tttgaaatcc 480  
 gccaggaag ttgaacttct acgtgaaaaa gtatatgccg ctttggaaga atatactaga 540  
 acaacacatc ccgatgaacc aggaagattt gcaaaacttt tgcttcgtct gccttcttta 600  
 cgttccatag gccttaagtg tttggagcat ttgtttttct ttcgccttat tggagatgtt 660  
 ccaattgata cgttcctgat ggagatgctt gaatcacctt ctgattcata a 711

<210> 46  
 <211> 236  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Chimeric RXR ligand binding domain

<400> 46

Ala Asn Glu Asp Met Pro Val Glu Lys Ile Leu Glu Ala Glu Leu Ala  
 1 5 10 15

Val Glu Pro Lys Thr Glu Thr Tyr Val Glu Ala Asn Met Gly Leu Asn  
 20 25 30

Pro Ser Ser Pro Asn Asp Pro Val Thr Asn Ile Cys Gln Ala Ala Asp  
 35 40 45

Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro His Phe  
 50 55 60

Ser Glu Leu Pro Leu Asp Asp Gln Val Ile Leu Leu Arg Ala Gly Trp  
 65 70 75 80

Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Ile Ala Val Lys  
 85 90 95

Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Asn Ser Ala  
 100 105 110

His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg Val Leu Thr Glu Leu  
 115 120 125

Val Ser Lys Met Arg Asp Met Gln Met Asp Lys Thr Glu Leu Gly Cys  
 130 135 140

Leu Arg Ser Val Ile Leu Phe Asn Pro Glu Val Arg Gly Leu Lys Ser  
 145 150 155 160

Ala Gln Glu Val Glu Leu Leu Arg Glu Lys Val Tyr Ala Ala Leu Glu  
 165 170 175

Glu Tyr Thr Arg Thr Thr His Pro Asp Glu Pro Gly Arg Phe Ala Lys  
 180 185 190

Leu Leu Leu Arg Leu Pro Ser Leu Arg Ser Ile Gly Leu Lys Cys Leu  
 195 200 205

Glu His Leu Phe Phe Phe Arg Leu Ile Gly Asp Val Pro Ile Asp Thr  
 210 215 220

Phe Leu Met Glu Met Leu Glu Ser Pro Ser Asp Ser  
 225 230 235

<210> 47

<211> 441

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 47

atgaagctac tgtcttctat cgaacaagca tgcgatattt gccgacttaa aaagctcaag 60

tgctccaaag aaaaaccgaa gtgcgccaaag tgtctgaaga acaactggga gtgtcgctac 120

tctcccaaaa ccaaaaggct tccgctgact agggcacatc tgacagaagt ggaatcaagg 180

ctagaaagac tggaacagct atttctactg atttttcctc gagaagacct tgacatgatt 240  
 ttgaaaatgg attctttaca ggatataaaa gcattgttaa caggattatt tgtacaagat 300  
 aatgtgaata aagatgccgt cacagataga ttggcttcag tggagactga tatgcctcta 360  
 acattgagac agcatagaat aagtgcgaca tcatcatcgg aagagagtag taacaaaggt 420  
 caaagacagt tgactgtatc g 441

<210> 48  
 <211> 147  
 <212> PRT  
 <213> *Saccharomyces cerevisiae*

<400> 48

Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu  
 1 5 10 15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu  
 20 25 30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro  
 35 40 45

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu  
 50 55 60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile  
 65 70 75 80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu  
 85 90 95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala  
 100 105 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser  
 115 120 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu  
 130 135 140

Thr Val Ser  
 145

<210> 49  
 <211> 606  
 <212> DNA

&lt;213&gt; Escherichia coli

&lt;400&gt; 49

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atgaaagcgt taacggccag gcaacaagag gtgtttgatc tcatccgtga tcacatcagc      60
cagacaggta tgccgccgac gcgtgcggaa atcgcgacgc gtttgggggtt ccgttcccca    120
aacgcggtctg aagaacatct gaaggcgctg gcacgcaaag gcgttattga aattgtttcc    180
ggcgcatcac gcgggattcg tctgttcgag gaagaggaag aagggttgcc gctggtaggt    240
cgtgtggctg ccggtgaacc acttctggcg caacagcata ttgaaggta ttatcaggtc    300
gatccttcct tattcaagcc gaatgctgat ttctgtctgc gcgtcagcgg gatgtcgatg    360
aaagatatcg gcattatgga tgggtgacttg ctggcagtgc ataaaactca ggatgtacgt    420
aacggtcagg tcgttgctgc acgtattgat gacgaagtta ccgttaagcg cctgaaaaaa    480
cagggcaata aagtcgaact gttgccagaa aatagcgagt ttaaaccaat tgtcgtagat    540
cttcgtcagc agagcttcac cattgaaggg ctggcggttg gggttattcg caacggcgac    600
tggtctg                                           606

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&lt;210&gt; 50

&lt;211&gt; 202

&lt;212&gt; PRT

&lt;213&gt; Escherichia coli

&lt;400&gt; 50

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Met Lys Ala Leu Thr Ala Arg Gln Gln Glu Val Phe Asp Leu Ile Arg
1              5              10              15

```

```

Asp His Ile Ser Gln Thr Gly Met Pro Pro Thr Arg Ala Glu Ile Ala
20              25              30

```

```

Gln Arg Leu Gly Phe Arg Ser Pro Asn Ala Ala Glu Glu His Leu Lys
35              40              45

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Ala Leu Ala Arg Lys Gly Val Ile Glu Ile Val Ser Gly Ala Ser Arg
50              55              60

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Gly Ile Arg Leu Leu Gln Glu Glu Glu Gly Leu Pro Leu Val Gly
65              70              75              80

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Arg Val Ala Ala Gly Glu Pro Leu Leu Ala Gln Gln His Ile Glu Gly
85              90              95

```

```

His Tyr Gln Val Asp Pro Ser Leu Phe Lys Pro Asn Ala Asp Phe Leu
100              105              110

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Leu Arg Val Ser Gly Met Ser Met Lys Asp Ile Gly Ile Met Asp Gly  
 115 120 125

Asp Leu Leu Ala Val His Lys Thr Gln Asp Val Arg Asn Gly Gln Val  
 130 135 140

Val Val Ala Arg Ile Asp Asp Glu Val Thr Val Lys Arg Leu Lys Lys  
 145 150 155 160

Gln Gly Asn Lys Val Glu Leu Leu Pro Glu Asn Ser Glu Phe Lys Pro  
 165 170 175

Ile Val Val Asp Leu Arg Gln Gln Ser Phe Thr Ile Glu Gly Leu Ala  
 180 185 190

Val Gly Val Ile Arg Asn Gly Asp Trp Leu  
 195 200

<210> 51

<211> 271

<212> DNA

<213> herpes simplex virus 7

<400> 51

atggggcccta aaaagaagcg taaagtcgcc ccccgaccg atgtcagcct gggggacgag 60  
 ctccacttag acggcgagga cgtggcgatg gcgcatgccg acgcgctaga cgatttcgat 120  
 ctggacatgt tgggggacgg ggattccccg gggccgggat ttacccccca cgactccgcc 180  
 ccctacggcg ctctggatat ggccgacttc gagtttgagc agatgtttac cgatgccctt 240  
 ggaattgacg agtacggtgg ggaattccccg g 271

<210> 52

<211> 90

<212> PRT

<213> herpes simplex virus 7

<400> 52

Met Gly Pro Lys Lys Lys Arg Lys Val Ala Pro Pro Thr Asp Val Ser  
 1 5 10 15

Leu Gly Asp Glu Leu His Leu Asp Gly Glu Asp Val Ala Met Ala His  
 20 25 30

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Asp Gly Asp  
 35 40 45

Ser Pro Gly Pro Gly Phe Thr Pro His Asp Ser Ala Pro Tyr Gly Ala



Tyr Asp Val Pro Asp Tyr  
100

<210> 55  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> GAL4 response element

<400> 55  
ggagtactgt cctccgagc

19

<210> 56  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 2xLexAop response element

<400> 56  
ctgctgtata taaaaccagt gggtatatgt acagta

36

<210> 57  
<211> 334  
<212> PRT  
<213> Choristoneura fumiferana

<400> 57

Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys Arg Lys Glu  
1 5 10 15

Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser Thr Thr Thr  
20 25 30

Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro Pro Pro Pro  
35 40 45

Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu Ser Asp Lys  
50 55 60

Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu Thr Ala Asn  
65 70 75 80

Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp Gly Tyr Glu  
85 90 95

Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr Trp Gln Gln  
100 105 110

Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg Gln Ile Thr  
 115 120 125

Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly  
 130 135 140

Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile Thr Leu Leu  
 145 150 155 160

Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala Arg Arg Tyr  
 165 170 175

Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln Ala Tyr Thr  
 180 185 190

Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile Glu Asp Leu  
 195 200 205

Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp Asn Ile His  
 210 215 220

Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg Pro Gly Leu  
 225 230 235 240

Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr Leu Asn Thr  
 245 250 255

Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala Arg Ser Ser  
 260 265 270

Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu Arg Thr Leu  
 275 280 285

Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu Lys Asn Arg  
 290 295 300

Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala Asp Met Ser  
 305 310 315 320

His Thr Gln Pro Pro Pro Ile Leu Glu Ser Pro Thr Asn Leu  
 325 330

<210> 58  
 <211> 549  
 <212> PRT

&lt;213&gt; Drosophila melanogaster

&lt;400&gt; 58

Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg Arg  
 1 5 10 15

Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Met Thr Thr Ser Pro Ser  
 20 25 30

Ser Gln His Gly Gly Asn Gly Ser Leu Ala Ser Gly Gly Gly Gln Asp  
 35 40 45

Phe Val Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu Pro Pro Gln  
 50 55 60

His Ala Thr Ile Pro Leu Leu Pro Asp Glu Ile Leu Ala Lys Cys Gln  
 65 70 75 80

Ala Arg Asn Ile Pro Ser Leu Thr Tyr Asn Gln Leu Ala Val Ile Tyr  
 85 90 95

Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Glu Glu Asp  
 100 105 110

Leu Arg Arg Ile Met Ser Gln Pro Asp Glu Asn Glu Ser Gln Thr Asp  
 115 120 125

Val Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr Val Gln Leu  
 130 135 140

Ile Val Glu Phe Ala Lys Gly Leu Pro Ala Phe Thr Lys Ile Pro Gln  
 145 150 155 160

Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met  
 165 170 175

Leu Arg Met Ala Arg Arg Tyr Asp His Ser Ser Asp Ser Ile Phe Phe  
 180 185 190

Ala Asn Asn Arg Ser Tyr Thr Arg Asp Ser Tyr Lys Met Ala Gly Met  
 195 200 205

Ala Asp Asn Ile Glu Asp Leu Leu His Phe Cys Arg Gln Met Phe Ser  
 210 215 220

Met Lys Val Asp Asn Val Glu Tyr Ala Leu Leu Thr Ala Ile Val Ile

225	230	235	240
Phe Ser Asp Arg Pro Gly Leu Glu Lys Ala Gln Leu Val Glu Ala Ile	245	250	255
Gln Ser Tyr Tyr Ile Asp Thr Leu Arg Ile Tyr Ile Leu Asn Arg His	260	265	270
Cys Gly Asp Ser Met Ser Leu Val Phe Tyr Ala Lys Leu Leu Ser Ile	275	280	285
Leu Thr Glu Leu Arg Thr Leu Gly Asn Gln Asn Ala Glu Met Cys Phe	290	295	300
Ser Leu Lys Leu Lys Asn Arg Lys Leu Pro Lys Phe Leu Glu Glu Ile	305	310	315
Trp Asp Val His Ala Ile Pro Pro Ser Val Gln Ser His Leu Gln Ile	325	330	335
Thr Gln Glu Glu Asn Glu Arg Leu Glu Arg Ala Glu Arg Met Arg Ala	340	345	350
Ser Val Gly Gly Ala Ile Thr Ala Gly Ile Asp Cys Asp Ser Ala Ser	355	360	365
Thr Ser Ala Ala Ala Ala Ala Ala Gln His Gln Pro Gln Pro Gln Pro	370	375	380
Gln Pro Gln Pro Ser Ser Leu Thr Gln Asn Asp Ser Gln His Gln Thr	385	390	395
Gln Pro Gln Leu Gln Pro Gln Leu Pro Pro Gln Leu Gln Gly Gln Leu	405	410	415
Gln Pro Gln Leu Gln Pro Gln Leu Gln Thr Gln Leu Gln Pro Gln Ile	420	425	430
Gln Pro Gln Pro Gln Leu Leu Pro Val Ser Ala Pro Val Pro Ala Ser	435	440	445
Val Thr Ala Pro Gly Ser Leu Ser Ala Val Ser Thr Ser Ser Glu Tyr	450	455	460
Met Gly Gly Ser Ala Ala Ile Gly Pro Ile Thr Pro Ala Thr Thr Ser	465	470	475
			480

Ser Ile Thr Ala Ala Val Thr Ala Ser Ser Thr Thr Ser Ala Val Pro  
 485 490 495

Met Gly Asn Gly Val Gly Val Gly Val Gly Val Gly Gly Asn Val Ser  
 500 505 510

Met Tyr Ala Asn Ala Gln Thr Ala Met Ala Leu Met Gly Val Ala Leu  
 515 520 525

His Ser His Gln Glu Gln Leu Ile Gly Gly Val Ala Val Lys Ser Glu  
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His Ser Thr Thr Ala  
 545

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 <211> 1288  
 <212> DNA  
 <213> Choristoneura fumiferana

<400> 59  
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 cccttcgcc agatcacaga gatgactatc ctcacggtcc aacttatcgt ggagtccgcg 660  
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 <211> 309  
 <212> DNA  
 <213> Simian virus 40

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 tgcatctcaa ttagtcagca accatagtcc cgcccctaac tccgcccata ccgcccctaa 180  
 ctccgccagc ttccgcccat tctccgcccc atggctgact aatttttttt atttatgcag 240  
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 gcctaggct 309

<210> 61  
 <211> 24  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic E1b minimal promoter

<400> 61  
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<210> 62  
 <211> 1653  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> luciferase gene

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 gcttttacag atgcacatat cgagggtgaac atcacgtacg cggaataactt cgaaatgtcc 180  
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 aaaaaattac caataatcca gaaaattatt atcatggatt ctaaaacgga ttaccaggga 480  
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 tttgtaccag agtcctttga tcgtgacaaa acaattgcac tgataatgaa ttcctctgga 600  
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 aaggccaaga agggcggaaa gtccaaattg taa 1653

<210> 63  
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 <212> DNA  
 <213> Mus musculus

<400> 63  
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18

<210> 64  
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 <212> DNA  
 <213> Locusta migratoria

&lt;400&gt; 64

tcaccttctg attcataa

18

&lt;210&gt; 65

&lt;211&gt; 1054

&lt;212&gt; DNA

&lt;213&gt; Choristoneura fumiferana

&lt;400&gt; 65

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atgcagtgtg aacctccacc tcctgaagca gcaaggattc acgaagtggc cccaagggtt 180

ctctccgaca agctgttggg gacaaaccgg cagaaaaaca tccccagtt gacagccaac 240

cagcagttcc ttatcgccag gctcatctgg taccaggacg ggtacgagca gccttctgat 300

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gacagtgttc tgttcgcgaa caaccaagcg tacactcgcg acaactaccg caaggctggc 600

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atcgccgatg ccgcgtccgg ccgcgtgct ctga 1054

&lt;210&gt; 66

&lt;211&gt; 798

&lt;212&gt; DNA

&lt;213&gt; Choristoneura fumiferana

&lt;400&gt; 66

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cgttaccgag cgcccgctct ctccctctgc caaataggca acaagcaaata agcggcggtg 180

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gctctccgct ccctctcgct caagagcttc gaacacctct acttcttcca cctcgtggcc    720
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<210> 67
<211> 1650
<212> DNA
<213> Drosophila melanogaster

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<400> 67
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ttggcctctg gtggcgccca agactttgtt aagaaggaga ttcttgacct tatgacatgc    180
gagccgcccc agcatgccac tattccgcta ctacctgatg aaatattggc caagtgtcaa    240
gcgcgcaata taccttcctt aacgtacaat cagttggccg ttatatacaa gttaatttgg    300
taccaggatg gctatgagca gccatctgaa gaggatctca ggcgtataat gagtcaacct    360
gatgagaacg agagccaaac ggacgtcagc ttccggcata taaccgagat aaccatactc    420
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gaggaccaga tcacgttact aaaggcctgc tcgtcggagg tgatgatgct gcgtatggca    540
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gagatgtgtt tctcactaaa gctcaaaaac cgcaactgct ccaagttcct cgaggagatc    960
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gttgagctcg gtgttggggg gggcggaac gtcagcatgt atgcgaacgc ccagacggcg 1560
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gttaagtcgg agcactcgac gactgcatag 1650

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<210> 68
<211> 1586
<212> DNA
<213> Bamecia argentifoli

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tccagtgtgc tgtgaagcga aaagagaaaa aagcgcaaaa ggacaaagat aaacctaact 540
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gttttgacaa actaattcgt gaagatcaaa tagctttatt aaaggcatgt agtagtgaag 900
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ctaaccagcc gtatacgaga gaatcataca ctgtagctgg catgggtgat actgtggagg	1020
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gcaaaaaaaaa aagcggccgc gaattc	1586

&lt;210&gt; 69

&lt;211&gt; 1109

&lt;212&gt; DNA

<213> *Nephotetix cincticeps*

&lt;400&gt; 69

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cgagtgaaac ctcgcagtcg gaccatcttc gccaaactgc tctccgttct caccgagctg 1020  
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<210> 70  
 <211> 401  
 <212> PRT  
 <213> Choristoneura fumiferana

<400> 70

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 20 25 30

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Met Arg Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Ala Val  
 50 55 60

Gly Met Arg Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys  
 65 70 75 80

Arg Lys Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser  
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Thr Thr Thr Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro  
 100 105 110

Pro Pro Pro Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu  
 115 120 125

Ser Asp Lys Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu  
 130 135 140

Thr Ala Asn Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp  
 145 150 155 160

Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr  
 165 170 175

Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg  
 180 185 190

Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe  
 195 200 205

Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile  
 210 215 220

Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala  
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Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln  
 245 250 255

Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile  
 260 265 270

Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp  
 275 280 285

Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg  
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Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr  
 305 310 315 320

Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala  
 325 330 335

Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu  
 340 345 350

Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu  
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Lys Asn Arg Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala  
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Asp Met Ser His Thr Gln Pro Pro Pro Ile Leu Glu Ser Pro Thr Asn  
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Leu

<210> 71  
 <211> 894  
 <212> DNA

&lt;213&gt; Tenebrio molitor

&lt;400&gt; 71

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&lt;210&gt; 72

&lt;211&gt; 298

&lt;212&gt; PRT

&lt;213&gt; Tenebrio molitor

&lt;400&gt; 72

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Arg Pro Glu Cys Val Val Pro Glu Val Gln Cys Ala Val Lys Arg Lys
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Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Pro Asn Ser Thr Thr Asn
20              25              30

```

```

Gly Ser Pro Asp Val Ile Lys Ile Glu Pro Glu Leu Ser Asp Ser Glu
35              40              45

```

```

Lys Thr Leu Thr Asn Gly Arg Asn Arg Ile Ser Pro Glu Gln Glu Glu
50              55              60

```

```

Leu Ile Leu Ile His Arg Leu Val Tyr Phe Gln Asn Glu Tyr Glu His
65              70              75              80

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Pro Ser Glu Glu Asp Val Lys Arg Ile Ile Asn Gln Pro Ile Asp Gly  
                             85                            90                            95

Glu Asp Gln Cys Glu Ile Arg Phe Arg His Thr Thr Glu Ile Thr Ile  
                             100                            105                            110

Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Arg Leu Pro Gly Phe  
                             115                            120                            125

Asp Lys Leu Leu Gln Glu Asp Gln Ile Ala Leu Leu Lys Ala Cys Ser  
                             130                            135                            140

Ser Glu Val Met Met Phe Arg Met Ala Arg Arg Tyr Asp Val Gln Ser  
                             145                            150                            155                            160

Asp Ser Ile Leu Phe Val Asn Asn Gln Pro Tyr Pro Arg Asp Ser Tyr  
                             165                            170                            175

Asn Leu Ala Gly Met Gly Glu Thr Ile Glu Asp Leu Leu His Phe Cys  
                             180                            185                            190

Arg Thr Met Tyr Ser Met Lys Val Asp Asn Ala Glu Tyr Ala Leu Leu  
                             195                            200                            205

Thr Ala Ile Val Ile Phe Ser Glu Arg Pro Ser Leu Ile Glu Gly Trp  
                             210                            215                            220

Lys Val Glu Lys Ile Gln Glu Ile Tyr Leu Glu Ala Leu Arg Ala Tyr  
                             225                            230                            235                            240

Val Asp Asn Arg Arg Ser Pro Ser Arg Gly Thr Ile Phe Ala Lys Leu  
                             245                            250                            255

Leu Ser Val Leu Thr Glu Leu Arg Thr Leu Gly Asn Gln Asn Ser Glu  
                             260                            265                            270

Met Cys Ile Ser Leu Lys Leu Lys Asn Lys Lys Leu Pro Pro Phe Leu  
                             275                            280                            285

Asp Glu Ile Trp Asp Val Asp Leu Lys Ala  
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Ser Lys Lys His Gln Lys Asp Arg Pro Asn Ser Thr Thr Arg Glu Ser  
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Pro Ser Ala Leu Met Ala Pro Ser Ser Val Gly Gly Val Ser Pro Thr  
 35 40 45

Ser Gln Pro Met Gly Gly Gly Gly Ser Ser Leu Gly Ser Ser Asn His  
 50 55 60

Glu Glu Asp Lys Lys Pro Val Val Leu Ser Pro Gly Val Lys Pro Leu  
 65 70 75 80

Ser Ser Ser Gln Glu Asp Leu Ile Asn Lys Leu Val Tyr Tyr Gln Gln  
                             85                            90                            95

Glu Phe Glu Ser Pro Ser Glu Glu Asp Met Lys Lys Thr Thr Pro Phe  
                             100                            105                            110

Pro Leu Gly Asp Ser Glu Glu Asp Asn Gln Arg Arg Phe Gln His Ile  
                             115                            120                            125

Thr Glu Ile Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ser Lys  
                             130                            135                            140

Arg Val Pro Gly Phe Asp Thr Leu Ala Arg Glu Asp Gln Ile Thr Leu  
                             145                            150                            155                            160

Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Gly Ala Arg Lys  
                             165                            170                            175

Tyr Asp Val Lys Thr Asp Ser Ile Val Phe Ala Asn Asn Gln Pro Tyr  
                             180                            185                            190

Thr Arg Asp Asn Tyr Arg Ser Ala Ser Val Gly Asp Ser Ala Asp Ala  
                             195                            200                            205

Leu Phe Arg Phe Cys Arg Lys Met Cys Gln Leu Arg Val Asp Asn Ala  
                             210                            215                            220

Glu Tyr Ala Leu Leu Thr Ala Ile Val Ile Phe Ser Glu Arg Pro Ser  
                             225                            230                            235                            240

Leu Val Asp Pro His Lys Val Glu Arg Ile Gln Glu Tyr Tyr Ile Glu  
                             245                            250                            255

Thr Leu Arg Met Tyr Ser Glu Asn His Arg Pro Pro Gly Lys Asn Tyr  
                             260                            265                            270

Phe Ala Arg Leu Leu Ser Ile Leu Thr Glu Leu Arg Thr Leu Gly Asn  
                             275                            280                            285

Met Asn Ala Glu Met Cys Phe Ser Leu Lys Val Gln Asn Lys Lys Leu  
                             290                            295                            300

Pro Pro Phe Leu Ala Glu Ile Trp Asp Ile Gln Glu  
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&lt;400&gt; 75

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